Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

Trinexapac-ethyl (ISO);

ethyl(1RS, 4EZ)4-[cyclopropyl(hydroxy)methylene]-3,5-dioxocyclohexanecarboxylate

EC Number:	**not allocated**
CAS Number:	**95266-40-3**
Index Number:	**607-RST-VW-Y**

Contact details for dossier submitter:

Environmental Protection Agency

A.Juozapavicius St 9

LT-09311 Vilnius, Lithuania

Version number: 2.0 **Date: March 2018**

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Version History

When	What
31 March 2017	First version of Draft Renewal Assessment Report (DRAR) submitted to EFSA
June 2017	First version of Assessment Report and Proposal for Harmonised Classification and Labelling (CLH report) submitted to ECHA
March 2018	Second version CLP report submitted to ECHA. This report has been revised following the outcome of the EU peer review of the pesticide risk assessment of the active substance trinexapac-ethyl.

The following sections are considered necessary for the harmonised classification and labelling according to the CLP criteria:

- **RAR Volume 3 B.2 (AS) Physical and chemical properties**
- **RAR Volume 3 B.6 (AS) Toxicology and metabolism data**
- **RAR Volume 3 B.8 (AS) Environmental fate and behaviour**
- **RAR Volume 3 B.9 (AS) Ecotoxicology**

LEVEL 1

1 Statement of subject matter and purpose for which this report has been prepared and background information on the application

1.1 Context in which the draft assessment report was prepared

1.1.1 Purpose for which the draft assessment report was prepared

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

1.1.3 EU Regulatory history for use in Plant Protection Products

1.1.4 Evaluations carried out under other regulatory contexts

1.2 Applicant(s) information

1.2.1 Name and address of applicant(s) for (renewal of) approval of the active substance

1.2.2 Producer or producers of the active substance

1.2.3 Information relating to the collective provision of dossiers

<u>**Applicant on behalf of the EU Task Force of trinexapac-ethyl**</u>

Syngenta Crop Protection AG European Product Registration B8.4.29 Scharzwaldalllee 2015 CH-4058 Basel Switzerland	<u>Contact:</u> [redacted]

Other Task Force members (in alphabetical order)

Adama Agriculture BV Eurofins Regulatory AG Weidenweg 15 CH-4310 Rheinfelden Switzerland	<u>Contact:</u> [redacted]
Cheminova A/S P.O. Box 9 DK-7620 Lemvig Denmark	<u>Contact:</u> [redacted]
Helm AG Nordkanalstrasse 28 20097 Hamburg Germany	<u>Contact:</u> [redacted]

1.3 Identity of the active substance

1.3.1 Common name proposed or ISO-accepted and synonyms

Trinexapac-ethyl

1.3.2 Chemical name (IUPAC and CA nomenclature)

IUPAC	ethyl (1*RS*,4*EZ*)-4-cyclopropyl(hydroxy)methylene-3,5-dioxocyclohexanecarboxylate[1]
CA	ethyl 4-(cyclopropylhydroxymethylene)-3,5-dioxocyclohexanecarboxylate

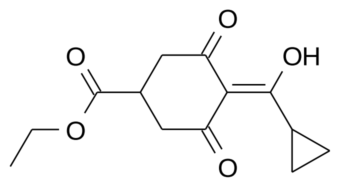
1.3.3 Producer's development code numbers

CGA 163935

1.3.4 CAS, EC and CIPAC numbers

CAS: 95266-40-3
EC: not allocated
CIPAC: 732.202

1.3.5 Molecular and structural formulae, molecular mass

Molecular formula	$C_{13}H_{16}O_5$
Structural formula	
Molecular mass	252.3 g/mol

1.3.6 Method of manufacture (synthesis pathway) of the active substance

Confidential information, see Volume 4 Annex C.

1.3.7 Specification of purity of the active substance in g/kg

Min.purity 950 g/kg.

1.3.8 Identity and content of additives (such as stabilisers) and impurities

1.3.8.1 Additives

Confidential information, see Volume 4 Annex C.

1.3.8.2 Significant impurities

Confidential information, see Volume 4 Annex C.

1.3.8.3 Relevant impurities

Toluene: max. 3 g/kg

Ethyl (1RS)-ethyl 3 hydroxy-5oxocyclohex-3-ene-1-carboxylate (CGA158377): 6 g/kg

Other potentially relevant impurities: Open

1.3.9 Analytical profile of batches

Confidential information, see Volume 4 Annex C.

[1] This is the revised IUPAC name of trinexapac-ethyl following the EU peer review of trinexapac-ethyl (EU agreed End points list, 2018). This name is the most correct as it reflects chirality and the *EZ* isomerism.

1.4 Information on the plant protection product

1.4.1 Applicant

Name: Syngenta Crop Protection AG (lead registrant)

(on behalf of the Trinexapac Task Force consisting of Syngenta Crop Protection AG, ADAMA Celsius B.V., Amsterdam (NL), Cheminova A/S and Helm AG according to Commission Regulation (EC) No. 844/2012 of 18 September 2012 for the renewal of the approval of an active substance under Commission Regulation (EC) No. 1107/2009)

Address: Schwarzwaldalle
P.O.Box
CH-4002 Basel
Switzerland

1.4.2 Producer of the plant protection product

Name: Syngenta Crop Protection AG (lead registrant)

Address: Schwarzwaldalle
P.O.Box
CH-4002 Basel
Switzerland

1.4.3 Trade name or proposed trade name and producer's development code number of the plant protection product

Trade name: Moddus ME
A8587F

1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product

1.4.4.1 Composition of the plant protection product

1.4.4.2 Information on the active substances

250 g/L trinexapac ethyl (26.4 % w/w)

1.4.4.3 Information on safeners, synergists and co-formulants

Confidential information, see Volume 4 Annex C

1.4.5 Type and code of the plant protection product

micro-emulsion, ME

1.4.6 Function

Plant growth regulator

1.4.7 Field of use envisaged

Agriculture

1.4.8 Effects on harmful organisms

Trinexapac-ethyl belongs to the chemical group, cyclohexanediones and is taken up by plants almost exclusively through the green portions of the plant. Uptake by the plant is rapid and quickly followed by transport in to the active meristem tissues. The growth regulatory activity is expressed in these tissues as an inhibition of internode elongation.

In contrast to members of the cyclohexanediones group that are herbicidal, trinexapac-ethyl does not influence the fatty acid metabolism of plants. Trinexapac-ethyl is a gibberellin antagonist, and is therefore similar to other plant growth regulators such as the triazoles and Chlormequat. However, in contrast to other gibberellin antagonists used commonly in crop management, trinexapac-ethyl does not inhibit the enzyme, Kaurenoxidase, which is active in the initial steps of Gibberellic acid synthesis.

A more exact determination of the mode-of-action for trinexapac-ethyl was made using barley as a model plant system. Trinexapac-ethyl inhibits later stages in the synthetic pathway for Gibberellin. After application of trinexapac-ethyl, the amount of active Gibberellic acid in the test plants reduces due to the blocking of hydroxylation of GA20 to the hormonally active GA1. The inhibitory action of trinexapac-ethyl for this enzymatic hydroxylation can be confirmed in vitro.

Through this inhibition of Gibberellic acid synthesis, the elongation of shoots is reduced and the height of the plant, dependent on application timing, is reduced.

1.5 Detailed uses of the plant protection product (to be included for each preparation for which documentation was submitted)

1.5.1 Details of representative uses

A8587F is a foliar active plant growth regulator in cereals to prevent lodging. Details of the intended uses are provided in the table 1.5.1-1.

Table 1.5.1-1: DETAILS OF INTENDED USES AND CONDITIONS OF USE

Tradename: A8587F
Active substance: Trinexapac-ethyl

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)**	**Application**				**Application rate**			**PHI (days)**	**Remarks:**
					Method / Kind	**Timing / Growth stage of crop & season**	**Max. number a) per use b) per crop/ season**	**Min. interval between applications (days)**	**L product / ha a) max. rate per appl. b) max. total rate per crop/season**	**kg as/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		**e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures**
1	EU	Barley, winter	F	Prevention of lodging	foliar spray	25-49	1	-	0.8	200	100-400	n.a.	
2	EU	Barley, spring	F	Prevention of lodging	foliar spray	25-37	1	-	0.6	150	100-400	n.a.	
3	EU	Wheat, winter	F	Prevention of lodging	foliar spray	25-49	1	-	0.5	125	100-400	n.a.	

1.5.2 Further information on representative uses

Method of Application

The method of application is by spray application using a hydraulic vehicle-mounted spray equipment with a water volume generally of 100-400 L/ha.

Number and Timings of Applications and Duration of Protection

Maximum number of applications and their timings: One application per crop/season.

Growth stages of crops or plants to be protected: between BBCH 25 and 49 in winter barley and winter wheat or between BBCH 25 and 37 in spring barley.

Development stages of the harmful organism concerned: Not applicable.

Inhibition of plant growth: From experimental and practical use it is known that trinexapac-ethyl can inhibit plant growth of cereals for a period of 10-20 days.

Duration of protection afforded by the maximum number of applications: Not applicable (only one application).

Refer to Table 3.3-1 for further details.

Necessary Waiting Periods or Other Precautions to Avoid Phytotoxic Effects on Succeeding Crops

Minimum waiting periods or other precautions between last application and sowing or planting succeeding crops: The active substance, trinexapac-ethyl, is rapidly metabolised in soil, primarily through hydrolytic – microbial processes causing hydrolysis of the ester bonds forming an acid metabolite (CGA179 500). This acid metabolite is also quickly broken down through a number of short-lived, polar metabolites. The ring structures of the active molecule are fully mineralized and a significant portion is released as CO2. Given the very short half-life of the active ingredient and its primary metabolite in soil, coupled with the lack of significant root uptake, no effect on succeeding crops is to be expected.

Limitations on choice of succeeding crops: No effect of A8587F on following crops is to be expected when applied at recommended rates.

Proposed Instructions for Use

Proposed instructions for use as printed on labels are not relevant to this application. However national labels can be provided on request.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

Details of the additional intended uses are provided in the table 1.5.3-1.

Table 1.5.3-1: Summary of additional intended uses for which MRL applications have been made, that in addition to the uses above, have also been considered in the consumer risk assessment (*name of active substance or the respective variant*)

Regulation (EC) N° 1107/2009 Article 8.1(g))

Important note: efficacy, environmental risk and risk to humans by exposure other than via their diet have not been assessed for these uses

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
MRL Application (according to Article 8.1(g) of Regulation (EC) No 1107/2009)															
Rye	*EU*	*Moddus Evo*	*F*	*Prevention of lodging*	*DC*	*250 g/L*	*foliar spray*	*25-49*	*1*	-	*0.5*	*100-400*	*0.125*	-	*No trials provided. Extrapolation from wheat*

DC – dispersible concentrate formulation

1.5.4 Overview on authorisations in EU Member States

Trinexapac-ethyl containing products are authorised in many Member States.

The applicant has provided information on Trinexapac-ethyl authorisation in EU Member States (please refer to supporting document D2 in the Dossier).

The representative formulation A8587F is authorised in Austria, France, Germany and Switzerland.

LEVEL 2

2 Summary of active substance hazard and of product risk assessment

2.1 Identity

2.1.1 Summary of identity

Trinexapac-ethyl is unclassified plant growth regulator.

Trinexapac is the ISO common name for (1*RS*, 4*EZ*)- 4-cyclopropyl(hydroxy)methylene-3,5-dioxocyclohexanecarboxylic acid (IUPAC). Due to the fact that the ethyl ester, a variant of trinexapac, is used in the formulated product, it should be noted that the evaluated data belong to the variant trinexapac-ethyl, unless otherwise specified *(ref. EFSA Scientific Report (2018)).*

The minimum purity of trinexapac-ethyl as manufactured should not be less than 950 g/kg (in comparison to 940 g/kg for Annex I inclusion of the active substance).

The FAO specification for active substance trinexapac-ethyl currently does not exist.

It should be noted that the specification for the technical material with respect to the maximum content of the impurities had been regarded as provisional at the time of the peer review process for trinexapac-ethyl Annex I inclusion. It had been considered that the proposed specification (max values) for non-relevant impurities were above the values declared in the technical material used for some toxicological and ecotoxicological tests. For the renewal of trinexapac- ethyl approval the amended specification supported by analytical profile of batches have been submitted for the EU re-assessment of trinexapac-ethyl.

2.2 Physical and chemical properties

2.2.1 Summary of physical and chemical properties of the active substance

Trinexapac-ethyl at room temperature is a solid without any explosive or oxidizing properties.

Most of the data concerning the physical and chemical properties had been assessed during the first evaluation for Annex I inclusion of trinexapac-ethyl (Directive 91/414/EEC) in 2007 and some new studies assessed in the view of Annex I renewal.

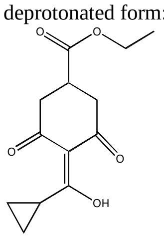
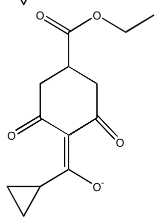
The pure active substance at room temperature is a solid with the melting point of 36.1 - 36.6°C and decomposition is starting at 310°C. A vapour pressure of trinexapac-etyl is 2.16×10^{-3} Pa at 25°C and the Henry's law constant was calculated to be 5.4×10^{-4} Pa.m^3 / mol, that indicates the active substance does not volatilise from water. The pK_a-value of the active substance is 4.57. Due to acidic properties the water solubility increases from 1.1 g / L at pH 3.5 to 21.1 g / L at pH 8.2 at 25°C. The log P_{ow} was determined to be - 0.29 at the neutral pH, indicating a low potential for the tested substance bioaccumulation. Trinexapac-ethyl is an ester. It had been determined that trinexapac-ethyl is hydrolytically more stable in acid/neutral than in a basic environment. It degrades at 25°C with half-lives between 460 and 8.1 days in the pH-range 5 to 9. At 20°C at pH 7 a photochemical half-life of 6.5 days was observed upon irradiation with Xenon arc light. These studies indicated that hydrolysis and photolysis are of importance in the degradation of trinexapac-ethyl in the environment.

Flammability, auto flammability, oxidizing and explosive properties of trinexapac-ethyl are not of concern and do not create critical problems in the production environment or during transport and storage of trinexapac-ethyl.

Table 1: **Summary of physicochemical properties of the active substance**

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Physical state at 20°C and 101,3 kPa	white powder	Das, 2000b	visual assessment (purity 99.6 %; 25°C)
	fine powder	Das, 2000c	visual assessment (96.8 % technical grade active substance at 25°C)
Melting/freezing point	36.1°C – 36.6°C	Das, 1998	measured
Boiling point	thermal decomposition	Das, 2000a	estimated: due to decomposition it

Property	Value	Reference	Comment (e.g. measured or estimated)
	starts at about 310°C at the reduced pressure (4.2 Pa) the active substance would boil at 99.8°C		was not possible to determine the boiling point at normal pressure the boiling point was taken from the report on vapour pressure curve below (Rordorf, 1990)
Relative density	1.31	Füldner, 2000	measured (OECD 109, air comparison pycnometer)
Vapour pressure	2.16 x 10^{-3} Pa (extrapolated, at 25°C)	Rordorf, 1990	extrapolated from fit of measurents between 38°C and 170°C.
Surface tension	55.5 mN/m (at 90% saturation in double distilled water at 20°C) 58.3 mN/m (1.0 g/L aqueous solution at 22.5°C)	Martin, 2000 O'Connor, 2014	measured (96.8 % technical grade active substance) measured (99.6 % purity of active substance)
Water solubility	1.1 g/L (pH 3.5; distilled/purified water) 2.8 g/L (pH 4.9; phthalate buffer) 10.2 g/L (pH 5.5; phosphate buffer) 21.1 g/L (pH 8.2; borax buffer)	Stulz, 1993 Rodler, 1990	measured (96.8 % technical grade active substance) measured (96.8 % technical grade active substance)
Partition coefficient n-octanol/water	uncorrected values: at pH 5.0: $\log P_{ow}$ = 1.5 (P_{ow} = 33 ± 0.84) at pH 6.9: $\log P_{ow}$ =- 0.29 (P_{ow} = 0.52 ± 0.013) at pH 8.9: $\log P_{ow}$ = - 2.1 (P_{ow} = 0.0085 ± 0.00053) corrected values: at pH 5.0: $\log P_{ow}$ = 2.1 (P_{ow} =120 ± 3.8) at pH 6.9: $\log P_{ow}$ = 2.0 (P_{ow} = 110 ±1.9) at pH 8.9: $\log P_{ow}$ = 2.3 (P_{ow} = 190 ±9.9)	Kettner, 1999	measured The uncorrected values: the method OECD 107 (EEC A8) determines the test substance CGA 163935 in an aqueous form as an acid, the sum of neutral [HA] and deprotonated [A^-] form. The corrected values were calculated in the study report excluding deprotonated form.
Flash point	156 ± 8 °C	Jackson, 2014	measured (purity 96.8 %)
Flammability	the substance did not propagate combustion and the burning time over 200 mm was not determined. Trinexapac-ethyl is not classified for flammability	Jackson, 2014	measured (purity 96.8%)

Property	Value	Reference	Comment (e.g. measured or estimated)
Explosive properties			
Self - ignition temperature	355^{o}C 330 ± 35^{o}C	Schurch, 1992a Jackson, 2014	measured (indicated for brownsh solidified melt, purity information not available) measured (purity 96.8 %)
Oxidising properties	Test sample mixture of trinexapac-ethyl had a mean pressure rise time much longer (timed out (90s)) than that observed for the nitric acid reference mixture.	Jackson, 2014	measured (purity 96.8 %)
Granulometry	NA	NA	NA
Solubility in organic solvents and identity of relevant degradation products	acetone: > 500 g/L; dichloromethane:>500g/L ethyl acetate: > 500 g/L; hexane: 45 g/L methanol: > 500 g/L 1-octanol: 420 g/L toluene: > 500 g/L	Stulz, 1998	measured (purity 96.8 %; 25^{o}C)
Dissociation constant	pK_a = 4.57 at 20°C Deprotonation of trinexapac-ethyl according to equation to the neutral and deprotonated form:   Neutral form is predominantely present at pH < 4.57, deprotonated form - at pH > 4.57	Jakel, 1990 Burkhard, 1999	measured
Viscosity	not applicable, the active substance is a solid	NA	NA
Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	IR, UV, MS and [1]H-NMR (in $CDCl_3$) spectra and support the active substance structure. Results from the UV spectra (in methanol solution): Neutral: λ = 240.2 nm ε = 9335 L/mol.cm; λ = 277.4 nm ε = 13976 L/mol.cm Acidic: λ = 240.0 nm ε = 11712 L/mol.cm λ = 280.4 nm ε = 12368 L/mol.cm Basic:	Roth, 1997	measured (purity 99.6 %)

Property	Value	Reference	Comment (e.g. measured or estimated)
	λ = 270.8 nm ε = 21320 L/mol.cm Absorbtion ends at about 320 nm, so at 290 nm ε is > 10 L/mol.cm. No further absorption is between 340 and 750 nm		

2.2.1.1 Evaluation of physical hazards

2.2.1.1.1 Explosives

Table 2: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC A14	The test substance did not explode when exposed to heat, mechanical shock or friction	trinexapac-ethyl purity 96.8 %	Jackson, 2014

2.2.1.1.1.1 Short summary and overall relevance of the provided information on explosive properties

Trinexapac-ethyl is not expected to be explosive. An examination of the chemical structure of trinexapac-ethyl concluded that it does not contain any of the bond groupings known to confer explosive properties. This assessment is considered relevant for classification and labeling for explosive properties.

2.2.1.1.1.2 Comparison with the CLP criteria

Based on the CLP Regulation (EC) No. 1272/2008, Point 2.1.4.3 a substance is not classified as explosive if there are no chemical groups associated with explosive properties present in the molecule. Based on inspection of its structure, trinexapac-ethyl does not contain chemical groups associated with explosive properties.

2.2.1.1.1 Conclusion on classification and labelling for explosive properties

Trinexapac-ethyl was also examined for explosive properties following the procedures specified in test method EEC A.14 and was found to be not explosive. Trinexapac-ethyl does not require classification as an explosive substance.

2.2.1.1.2 Flammable gases (including chemically unstable gases)

Table 3: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference
None	-	not applicable	-

2.2.1.1.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Not applicable. Trinexapac-ethyl is not a gas.

2.2.1.1.2.2 Comparison with the CLP criteria

Not applicable. Trinexapac-ethyl is not a gas.

2.2.1.1.2.3 Conclusion on classification and labelling for flammable gases

Not applicable. Trinexapac-ethyl is not a gas.

2.2.1.1.3 Oxidising gases

Table 4: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference
None	-	not applicable	-

2.2.1.1.3.1 Short summary and overall relevance of the provided information on oxidising gases
Not applicable. Trinexapac-ethyl is not a gas.

2.2.1.1.3.2 Comparison with the CLP criteria
Not applicable. Trinexapac-ethyl is not a gas.

2.2.1.1.3.3 Conclusion on classification and labelling for oxidising gases
Not applicable. Trinexapac-ethyl is not a gas.

2.2.1.1.4 Gases under pressure

Table 5: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference
None	-	not applicable	-

2.2.1.1.4.1 Short summary and overall relevance of the provided information on gases under pressure
Not applicable. Trinexapac-ethyl is not a gas.

2.2.1.1.4.2 Comparison with the CLP criteria
Not applicable. Trinexapac-ethyl is not a gas.

2.2.1.1.4.3 Conclusion on classification and labelling for gases under pressure
Not applicable. Trinexapac-ethyl is not a gas.

2.2.1.1.5 Flammable liquids

Table 6: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
None	-	not applicable	-

2.2.1.1.5.1 Short summary and overall relevance of the provided information on flammable liquids
Not applicable. Trinexapac-ethyl is not a liquid.

2.2.1.1.5.2 Comparison with the CLP criteria
Not applicable. Trinexapac-ethyl is not a liquid.

2.2.1.1.5.3 Conclusion on classification and labelling for flammable liquids
Not applicable. Trinexapac-ethyl is not a liquid.

2.2.1.1.6 Flammable solids

Table 7: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC Method A.10	Not flammable Preliminary test results: technical grade active substance did not propagate combustion and the burning time over 200 mm was not determined. Full test series were not required. Trinexapac-ethyl is not classified based on flash point or burning characteristics of the test substance: The flash point (ref. RAR B2.10/01 or Table 1 above) is above 55°C.	active substance at purity 96.8 %	Jackson, 2014

2.2.1.1.6.1 Short summary and overall relevance of the provided information on flammable solids

Trinexapac-ethyl is not a flammable solid. In laboratory testing, trinexapac ethyl technical material does not propagate combustion and therefore is not classified as highly flammable in terms of its burning characteristics. This data is considered reliable for classification and labelling for flammable solids.

2.2.1.1.6.2 Comparison with the CLP criteria

According to the CLP Regulation (EC) No. 1272/2008, Point 2.7.2.1, a substance is classified if in the burning rate test they exhibit a burning time <45 s or burning rate >2.2 mm/s. Trinexapac-ethyl did not exhibit a burning time above this cut-off criteria. The test substance melted and did not propagate the flame.

2.2.1.1.6.3 Conclusion on classification and labelling for flammable solids

Trinexapac-ethyl is not classified a highly flammable in terms of its burning characteristics.

2.2.1.1.7 Self-reactive substances

Table 8: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
EEC A2 OECD 103	Thermal decomposition starts at about 310 °C	pure active substance (99.6 %)	Das, 2000a

2.2.1.1.7.1 Short summary and overall relevance of the provided information on self-reactive substances

Trinexapac-ethyl is not self-reactive. Upon heating trinexapac-ethyl was unreactive until decomposed at about 310°C.

2.2.1.1.7.2 Comparison with the CLP criteria

According to the CLP Regulation (EC) No. 1272/2008, Point 2.8.4.2, if a test item has a decomposition point >75°C, the classification is not applicable. Trinexapac-ethyl has a decomposition point above this criteria.

2.2.1.1.7.3 Conclusion on classification and labelling for self-reactive substances

Not applicable, trinexapac-ethyl has a decomposition point >75°C.

2.2.1.1.8 Pyrophoric liquids

Table 9: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
None	-	not applicable	-

2.2.1.1.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids
Not applicable, trinexapac-ethyl is not a liquid.

2.2.1.1.8.2 Comparison with the CLP criteria
Not applicable, trinexapac-ethyl is not a liquid.

2.2.1.1.8.3 Conclusion on classification and labelling for pyrophoric liquids
Not applicable, trinexapac-ethyl is not a liquid.

2.2.1.1.9 Pyrophoric solids

Table 10: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
None	-	not applicable	

2.2.1.1.9.1 Short summary and overall relevance of the provided information on pyrophoric solids
Production or handling information shows that the substance does not ignite spontaneously on coming into contact with air.

2.2.1.1.9.2 Comparison with the CLP criteria
According to the CLP Regulation (EC) No. 1272/2008, Point 2.10.4.1, classification procedure for pyrophoric solids need not be applied when experience in production or handling shows that the substance does not ignite spontaneously on coming into contact with air at normal temperatures. Therefore classification for pyrophoric solids is not applicable to trinexapac-ethyl.

2.2.1.1.9.3 Conclusion on classification and labelling for pyrophoric solids
Trinexapac-ethyl is not classified a phyrophoric solid.

2.2.1.1.10 Self-heating substances

Table 11: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EEC A15 using IEC 60079-20-1 Test on self heating	auto ignition temperature is 330 ± 35 °C	active substance purity 96.8 %	Jackson, 2014

2.2.1.1.10.1 Short summary and overall relevance of the provided information on self-heating substances
Trinexapac-ethyl auto-ignition temperature is 330 ± 35 °C.

2.2.1.1.10.2 Comparison with the CLP criteria

According to the CLP Regulation (EC) No. 1272/2008, Point 2.11.1.1, a self-heating substance or mixture is a liquid or solid substance or mixture, other than a pyrophoric substance which, by reaction with air and without energy supply, is liable to self-heat.

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

Trinexapac-ethyl is not a self heating substance.

2.2.1.1.11 Substances which in contact with water emit flammable gases

Table 12: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
None	-	not applicable	-

2.2.1.1.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

Not applicable. The structure of trinexapac-ethyl does not contain any metals.

2.2.1.1.11.2 Comparison with the CLP criteria

According to the CLP Regulation (EC) No. 1272/2008, Point 2.12.4.1, the classification procedure need not be applied if chemical structure does not contain metallic element or metalloid element. Trinexapac-ethyl does not contain any metallic or metalloid elements.

2.2.1.1.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

The classification is not applicable based on the structure of trinexapac-ethyl.

2.2.1.1.12 Oxidising liquids

Table 13: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
EEC A21	The test sample of technical grade trinexapac-ethyl (which is solidified melt at room temperature) was liquefied in a hot water bath for testing. Test sample mixture of trinexapac-ethyl was found to have a mean pressure rise time much longer (timed out (90s)) than that observed for the nitric acid reference mixture.	active substance purity 96.8 %	Jackson, 2014

2.2.1.1.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Examination of the structure of trinexapac-ethyl indicates it is not an oxidizing substance. This is supported by the results of the oxidising testing following EC method A.21. This data is considered relevant for conclusions on the classification and labelling for oxidising solids. Trinexapac-ethyl is not classified as an oxidising substance.

2.2.1.1.12.2 Comparison with the CLP criteria

According to the CLP Regulation (EC) No. 1272/2008, point 2.14.4.1, a substance is not classified if it contains oxygen, fluorine or chlorine and these elements are chemically bounded only to carbon or hydrogen.

The structure of trinexapac ethyl meets this criteria, and trinexapac-ethyl is therefore not considered an oxidising substance.

2.2.1.1.12.3 Conclusion on classification and labelling for oxidising liquids
Not applicable, trinexapac-ethyl of technical grade is not an oxidising liquid.

2.2.1.1.13 Oxidising solids

Table 14: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
None	-	-	

2.2.1.1.13.1 Short summary and overall relevance of the provided information on oxidising solids
Examination of the structure of trinexapac-ethyl indicates it is not an oxidizing substance. This is supported by the results of the oxidising testing following EC method A.17 on the trinexapac technical grade active substance, solidified melt, above. This data is considered relevant for conclusions on the classification and labelling for oxidising substances. Trinexapac-ethyl is not classified as an oxidising substance.

2.2.1.1.13.2 Comparison with the CLP criteria
According to the CLP Regulation (EC) No. 1272/2008, point 2.14.4.1, a substance is not classified if it contains oxygen, fluorine or chlorine and these elements are chemically bounded only to carbon or hydrogen. The structure of trinexapac ethyl meets this criteria, and trinexapac-ethyl is therefore not considered an oxidising substance.

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids
Trinexapac-etyl is not classified as an oxidising substance.

2.2.1.1.14 Organic peroxides

Table 15: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference
None	-	not applicable	

2.2.1.1.14.1 Short summary and overall relevance of the provided information on organic peroxides
Not applicable. Trinexapac-ethyl is not an organic peroxide.

2.2.1.1.14.2 Comparison with the CLP criteria
According to the CLP Regulation (EC) No. 1272/2008, Point 2.15.1.1, organic peroxides are liquid or solid organic substances which contain the bivalent -O-O- structure and may be considered derivatives of hydrogen peroxide, where one or both of the hydrogen atoms have been replaced by organic radicals. There is no -O-O- moieties in the structure of trinexapac-ethyl.

2.2.1.1.14.3 Conclusion on classification and labelling for organic peroxides
Not applicable. Trinexapac-ethyl is not classified for organic peroxides.

2.2.1.1.15 Corrosive to metals

Table 16: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference

Method	Results	Remarks	Reference
None	-	not applicable	-

2.2.1.1.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No data is available.

2.2.1.1.15.2 Comparison with the CLP criteria

According to the CLP Regulation (EC) No. 1272/2008, Point 2.16.2.1, to be classified a substance must exhibit a corrosion rate on steel or aluminum surfaces exceeding 6.25 mm per year at a test temperature of 55°C. Based on storage stability testing with trinexapac-ethyl in contact with aluminium, the active substance was not observed to be corrosive to metal

2.2.1.1.15.3 Conclusion on classification and labelling for corrosive to metals

Conclusion could not be drawn based on the lack of data.

2.2.2 Summary of physical and chemical properties of the plant protection product

Plant protection product Moddus ME (A8587F) is a micro emulsion formulation without any explosive or oxidising properties. The formulation is classified as flammable liquid.

The product is a new representative formulation for Annex I renewal of trinexapac-ethyl.

The appearance of the product is an orange liquid with a sweetish, pungent odour. The pH of 1% aqueous emulsion of the formulation in deionised water is 3.5, its acidity was determined to be 4.83 % of sulphuric acid. The flash point of the formulation A 8587 F determined in closed cup by method EEC A9 was 44 ± 2^{o}C and therefore the formulation is a flammable liquid and needs too be classified as H226: flammable liquid category 3. The self-ignition temperature was determined to be 335^{o}C ± 2^{o}C and therefore the high self ignition temperature does not exhibit self heating properties.

The emulsion in water of A8587F was found to be surface active. The product is a Newtonian liquid, low viscosity formulation which does not satisfy the classification criteria for aspiration hazard. The overall stability data of the formulation (accelerated storage and storage at room temperature) indicate that the formulation remains stable for at least 2 years at ambient temperature when stored in original unopened HDPE containers. The technical characteristics of formulation A8587F are acceptable for micro-emulsion formulation according to the FAO/WHO manual for pesticide formulations.

Based on results of physical and chemical properties the product is not expected to create big problems in the environment or during transport and storage.

2.3 Data on application and efficacy

2.3.1 Summary of effectiveness

Trinexapac-ethyl acts as a plant growth regulator to prevent lodging and brackling (crop leaning) in field crops, like cereals, oil seed rape, pulses and grass seeds for seed production.

Trinexapac-ethyl is a late gibberellin (GA1) biosynthesis inhibitor and is taken up by plants almost exclusively through the green portions of the plant. The growth regulatory activity is expressed in these tissues as an inhibition of internode elongation.

2.3.2 Summary of information on the development of resistance

The development of resistance is not considered relevant due to the fact that trinexapac-ethyl effects a natural plant process by binding reversibly at the active site i.e. as the concentrations of the trinexapac-ethyl in the plant is reduced through metabolism, binding at the active site is also progressively reduced. Therefore the effect of trinexapac-ethyl is to produce a temporary effect within the plant, and in the absence of trinexapac-ethyl plant growth returns to normal.

2.3.3 Summary of adverse effects on treated crops

Trinexapac-ethyl containing products are authorised and used in EU for a long time. They are crop safe when used according to the label instructions.

2.3.4 Summary of observations on other undesirable or unintended side-effects

Minimum waiting periods or other precautions between last application and sowing or planting succeeding crops: The active substance, trinexapac-ethyl, is rapidly metabolised in soil, primarily through hydrolytic – microbial processes causing hydrolysis of the ester bonds forming an acid metabolite (CGA179 500). This acid metabolite is also quickly broken down through a number of short-lived, polar metabolites. The ring structures of the active molecule are fully mineralized and a significant portion is released as CO2. Given the very short half-life of the active ingredient and its primary metabolite in soil, coupled with the lack of significant root uptake, no effect on succeeding crops is to be expected.

Limitations on choice of succeeding crops: No effect of A8587F on following crops is to be expected when applied at recommended rates.

2.4 Further information

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

Sufficient information to address the respective data requirements is available (please refer to Vol. 3 CA B4 and Vol. 3 CP B4 for detailed information).

Active substance: trinexapac-ethyl

Hazard identification:

Health hazards:

Hazard Class and category Code: Skin Sens. 1B,

Hazard statement Code: H317

Pictogram, Signal Word Code: GHS07, Wng

Environmental hazards:

Warning. Very toxic to aquatic life with long lasting effects

Classification according to Regulation (EU) 1272/2008

Chronic aquatic toxicity Category 1 H410

Handling and Storage:

Store the product in closed original containers. Protect from light and humidity. Store separately from feed food and stimulants.

Precautions for safe handling

Hydrogen cyanide gas may be released during opening and dispensing.
Avoid breathing air from container headspace
When using do not eat, drink or smoke.
For personal protection recommendations for exposure controls relevant to the manufacture, formulation and packaging of trinexapac-etyl are given in the MSDS section 8.

Formulation A 8587F:

Keep containers tightly closed in a dry, cool and well-ventilated place.

Keep out of the reach of children.

Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

Keep in area equipped with sprinklers.

Keep away from food, drink and animal feeding stuffs.

Store in a well-ventilated place. Keep cool.

Use only in an area containing flame proof equipment.

Take precautionary measures against static discharges.

Advice on safe handling:

Avoid contact with skin and eyes.

When using, do not eat, smoke or drink.

If swallowed, seek medical advice immediately and show this container or label.

Use appropriate container to avoid environmental contamination.

Collect spillage

This material and its container must be disposed of in a safe way.

Dispose of containers to an approved waste disposal plant.

Incompatible materials: No substances are known which lead to the formation of hazardous substances or thermal reactions.

Transport information:

Active substance
Use unbreakable containers, make sure they cannot fall, and label in accordance with regulations.

Land transport (ADR/RID)

UN number:	UN 3077
Classification Rail / Road RID / ADR :	Class 9 Cipher 12C Kemmler Index 90 CEFIC No. 90G02
UN Proper shipping name :	environmentally hazardous substance, solid, N.O.S.
Additional information :	(trinexapac-ethyl)

Sea transport (IMDG**)**

UN number:	UN 3077
UN Proper shipping name :	environmentally hazardous substance, solid, N.O.S.
Transport hazard class(es)	9
Packaging group:	III
Labels:	9
Classification Sea IMDG-Code :	Not classified as dangerous good.
Environmental hazards:	*Marine pollutant*

Air transport (IATA –DGR)

UN number:	UN 3077
Classification Air ICAO / IATA :	Class 9 Packing group III
Proper shipping name :	environmentally hazardous substance, solid, N.O.S.
Additional information :	(trinexapac-ethyl)

Formulation A 8587F:

Land transport (ADR/ RID):

UN-Number:	UN 1105
UN Proper shipping name :	PENTANOLS SOLUTION
Transport hazard class(es):	3
Packaging group:	III
Labels:	3
Environmental hazards:	Environmentally hazardous
Tunnel restriction code:	D/E

Sea transport (IMDG):

UN-Number:	UN 1105
UN Proper shipping name :	PENTANOLS SOLUTION
Transport hazard class(es):	3
Packaging group:	III
Labels:	3
Environmental hazards:	Marine pollutant

Air transport (IATA-DGR) :

UN-Number:	UN 1105
UN Proper shipping name :	PENTANOLS SOLUTION
Transport hazard class(es):	3
Packaging group:	III
Labels:	3

Fire :

Active substance trinexapac-ethyl

Extinguishing media: Dry chemical extinguisher, foam, carbon dioxide or water spray (do not use direct jet of water)

Extinguishing media - small fires:
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.
Extinguishing media - large fires:
Alcohol-resistant foam or water spray
Do not use a solid water stream as it may scatter and spread fire.

<u>Combustion gases</u>: Trinexapac-ethyl contains the elements carbon, hydrogen and oxygen. In the event of fire the formation of carbon monoxide, carbon dioxide, must be anticipated.

Special hazrds arising from the substance or mixture

As the product contains combustible organic components, fire will produce dense black smoke containing hazardous products of combustion (see section 10[1]).
Exposure to decomposition products may be a hazard to health.

Advice to firefighters

Wear full protective clothing and self-contained breathing apparatus Do not allow run-off from fire fighting to enter drains or water courses
Cool closed containers exposed to fire with water spray.

Formulation A 8587F

Flammable liquid and vapour (hazard statement H226)

Hazardous components to be listed on the label: pentanol mixture of isomers

Special hazards arising from the substance or mixture:

[1] Section 10. Stability and reactivity: *10.6 Hydrogen cianide gas may develop in the headspace of containers at normal storage.*

	As the product contains combustible organic components, fire will produce dense black smoke containing hazardous products of combustion.
	Exposure to decomposition products may be a hazard to health.
	Flash back possible over considerable distance
Advice for firefighters:	Wear full protective clothing and self-contained breathing apparatus
	Do not allow run-off from fire fighting to enter drains or water courses.
	Cool closed containers exposed to fire with water spray.

Suitable extinguishing media:

Small fires: water spray, alcohol resistant foam, dry chemical or carbon dioxide.
Large fires: alcohol resistant foam.

Unsuitable extinguish media:

Shall not be used for safety reasons: Solid water stream (may scatter and spread fire).

Specific hazards during fire fighting:

During combustion, toxic and irritant vapours may be released.

As the product contains combustible organic components, fire will produce dense black smoke containing hazardous products of combustion. Exposure to decomposition products may be a hazard to health. Flash back possible over considerable distance.

Further information: Do not allow run-off from fire fighting to enter drains or water courses.

Cool closed containers exposed to fire with water spray.

Special protective equipment for fire fighters:

Wear full protective clothing and self-contained breathing apparatus.

2.4.2 Summary of procedures for destruction or decontamination

Active substance

Controlled incineration

The active substance trinexapac-ethyl can be disposed of safely by incineration in a modern incinerator, licensed to treat special contaminated waste, which fulfils the following conditions: temperature >800°C, minimum residence time within the incinerator: 2 seconds, equipped with a washing unit for flue gases. The ashes have to be disposed of at a suitable, approved waste disposal site. Wash water has to be disposed of via suitable wastewater treatment plant.

The active substance trinexapac-ethyl contains no halogens, therefore a formation of polyhalogenated dibenzo-p-dioxins and dibenzo-furans during incineration can be fully excluded. The reaction products are completely destroyed at temperatures above 800°C.

Incinerate at a licensed installation.

Formulation A8587 F

Incinerate at a licensed installation.

As the halogen content of A8587F is below the 60% trigger value, high temperature incineration is the preferred means of disposal for the active substances, formulated products, contaminated materials or contaminated packaging. Directive 96/47/EEC defines the controlled conditions for incineration.

Incineration should be carried out in a licensed incinerator operating at a temperature above 800°C and with a minimum gas phase residence time of two seconds.

<u>Disposal considerations</u>:

Waste treatment methods

Do not contaminate ponds, waterways or ditches with chemical or used container.
Do not dispose of waste into sewer.
Where possible recycling is preferred to disposal or incineration.
If recycling is not practicable, dispose of in compliance with local regulations.

Dispose the contaminated material at an authorised site.

Contaminated packaging

Empty remaining contents.
Triple rinse containers.
Empty containers should be taken to an approved waste handling site for recycling or disposal.
Do not re-use empty containers

2.4.3 Summary of emergency measures in case of an accident

Active substance

Fire fighting water has to be contained, concentrated and decontaminated by filtration using charcoal. The water can be disposed of at a suitable sewage treatment plant or incinerated. The charcoal can be disposed of in a suitable waste incineration plant in accordance with the official regulations.

First aid measures

If poisoning is suspected, immediately contact a physician, the nearest hospital, or the nearest Poison Control Centre. Tell the person contacted the complete product name, and the type and amount of exposure.

Ingestion:	Repeatedly administer medicinal charcoal in a large quantity of water. NOTE: Never give anything by mouth to an unconscious person. Do not induce vomiting. *If swallowed, seek medical advice immediately and show this container or label.* *Do NOT induce vomiting.*
Eye contact:	Rinse eyes with clean water for several minutes. *Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes* *Remove contact lenses.* *Immediate medical attention is required.*
Skin contact:	Remove contaminated clothing and thoroughly wash the affected parts of the body with soap and water. *Take off all contaminated clothing immediately.* *Wash off immediately with plenty of water.* *If skin irritation persists, call a physician.*

Wash contaminated clothing before re-use.

Inhalation: Immediately remove to fresh air.

Move the victim to fresh air.
If breathing is irregular or stopped, administer artificial respiration.
Keep patient warm and at rest.
Call a physician or poison control centre immediately.

Most important symptoms and effects, both acute and delayed

Symptoms: *No information available.*
Indication of any immediate medical attention and special treatment needed
Medical advice: *There is no specific antidote available.*
Treat symptomatically.

Formulation A 8587 F

Neutralisation procedure

Neutralisation is not an effective procedure for the destruction or decontamination of the formulation in case accidental spillage.
The spilled liquid should first be adsorbed onto a solid, such as sand, inert clay filler, saw dust or soil, before being swept up into a safe container to await disposal t an authorized site.

2.5 Methods of analysis

2.5.1 Methods used for the generation of pre-authorisation data

Validated methods for the determination of the active substance and the impurities in the technical material as manufactured are available. Validated analytical method (HPLC-UV) is available to determine the content of trinexapac-ethyl in the formulation A 8587F (Moddus ME).

Data generation methods for the determination of the residues of trinexapac-ethyl in products of plant and animal origin are available.

The two tables added below summarize the methods validated according to the criteria of SANCO/3029/99 rev.4 to support the studies for the risk assessment of trinexapac-ethyl.

Methods for the determination of the active substance and/or metabolites in products of plant origin

Commodities *(matrix group*)*	**Analyte**	**Method principle *LOQ***	**Reference**	**EU review**
tomato, apple (2) sunflower seed (3) barley grain (1) barley hay and straw	trinexapac (CGA179500)	HPLC-MS/MS *0.01 mg/kg*	GRM020.05A Hargreaves, 2008 supercedes REM 137.13 *validation:* Mayer, 2008, (ammended 2016)	New data Renewal
cereal grain (1) cereal straw dry broad beans (1)	trinexapac (CGA179500) free and conjugated forms	HPLC-MS/MS *0.01 mg/kg* *0.05 mg/kg*	GRM020.009A Braid & Tsui, 20156 GRM020.09A Braid & Tsui, 2016	New data Renewal

Commodities *(matrix group*)*	Analyte	Method principle *LOQ*	Reference	EU review
oilseed rape, seed (3) cereal grain (1) cereal straw		*0.01 mg/kg* *0.05 mg/kg* *0.02 mg/kg* *0.05 mg/kg*	GRM020.09B[1] Braid & Tsui, 2016 GRM020.16A[2] Braid & Tsui, 2016 *validation of GRM020.09B and GRM020.16A* Tsui, 2015	
grass forage grass straw grass seed seed screenings wheat grain (1) wheat forage wheat straw	trinexapac (CGA179500) free and conjugated forms	HPLC-MS/MS *0.01 mg/kg*	GRM020.01A (modified 110-01) *method and validation* Lin, 2008 *ILV* Thomas, 2010	New data Renewal
grass: forage hay straw seed and seed screenings	trinexapac (CGA179500)	HPLC-MS/MS *0.05 mg/kg*	110-01 *method and validation* Lin, 2002 *ILV* Cobin, Pyles, 2002	New data Renewal
grain (1) processed commodities: beer bread bran flour	CGA313458	HPLC-MS/MS *0.01 mg/kg*	GRM020.13A[3] Langridge, 2016 *validation* Langridge, 2016 (CEMR-7360-INT)	New data Renewal
processed commodities: beer bread	CGA113745	HPLC-MS/MS *0.01 mg/kg*	GRM020.14A[4] Langridge, 2016 *validation* Langridge, 2016 (CEMR-7360-INT)	New data Renewal
brewing and baking matrices: grain (1) beer bread bran flour	Cyclopropane carboxylic acid (CGA224439)	HPLC-MS/MS *0.01 mg/kg*	GRM020.15A Watson, 2016 *validation* Watson, 2016a	New data Renewal

*the numbers in brackets according to GD SANCO 825/00, 3.3. Commodities and four matrix groups: 1) dry commodities (high protein/high starch content) and commodities with high water content (2); high oil content (3), high acid content (4).

[1] [2]Method GRM020.09B indicated as update of GRM020.09A to include new validation data for dry broad beans and oilseed rape seeds.

[2] [2]Method GRM020.09B indicated as an update of GRM020.09A to include new validation data for dry broad beans and oilseed rape seeds.

[3] The method developed and validated for beer, bread, bran, wheat grain and flour.

[4] The method developed and validated for beer and bread. The method needs to be developed further and validated for bran, wheat grain and flour, due to low extractability in these matrices.

Methods for the determination of the active substance and/or metabolites in products of animal origin

Matrix	Analyte	Method principle ***LOQ***	Reference	EU review
muscle, fat, kidney, liver (bovine) and eggs (chicken) milk	trinexapac (CGA179500)	HPLC-MS/MS *0.01 mg/kg (tissues)* *0.005 mg/kg (milk)*	AGR/MOA/Trin-06 Sole, 2008; CHE/TRIN/08003	New data Renewal

2.5.2 Methods for post control and monitoring purposes

Methods for the determination of trinexapac-ethyl residues for the enforcement and monitoring purposes have not been completely available to fully cover all data requirements as stipulated in the Regulation (EC) 283/2013.

For the assessment of methods the following criteria have been applied:

- the mean recovery at each fortification level and for each sample matrix in the range of 70-110 % with a relative standard deviation of ≤ 20 %;
- no interfering blanks (<30 % LOQ);
- methods employ the simplest approach, involve lower costs, and require commonly available analytical techniques;
- methods are suitable to determine compounds of the residue definition;
- methods for plant and animal matrices as well as for drinking water are checked in an independent laboratory;
- the confirmation of methods has been addressed.

According to these criteria adequate analytical methods are listed in the tables below added for summary of the methods for the enforcement.

Methods for trinexapac acid (CGA179500) determination in food/feed of plant and animal origin

Commodity *(matrix group*)*	**Method**	**LOQ**	**Reference**	**EU review**
barley grain (1) lettuce (2) sunflower seed (3) barley hay and straw	HPLC-MS/MS monitoring 2 mass transitions	0.01 mg/kg 0.01 mg/kg 0.01 mg/kg 0.01 mg/kg	GRM020.05A Hargreaves, 2008 supercedes REM 137.13 *validation:* Mayer L, 2008, (ammended 2016)	New data Renewal[1]
wheat grain, dried broad bean (1) tomato, apple (2) sunflower seed (3) orange (4)	QuEChERS (LC-MS/MS) monitoring two mass transitions	0.01 mg/kg 0.01 mg/kg 0.01 mg/kg 0.01 mg/kg	Richter, 2015 Brown, 2015 (ILV)	New data Renewal

[1] The method GRM020.05A submitted as a method in support of wheat and barley residue trials (ref. Vol3 B5, B5.1.2.1 and data point KCA.4.1.2).
The method GRM020.05A considered to be *subsequent validation of method REM 137.13 which was already peer reviewed by the RMS Netherlands in 2005*, Addendum to the DAR.

Commodity *(matrix group*)*	Method	LOQ	Reference	EU review
milk eggs muscle liver fat	QuEChERS (LC-MS/MS) monitoring two mass transitions	0.01 mg/kg 0.01 mg/kg 0.01 mg/kg 0.01 mg/kg	Richter, 2015 *validation* Richter, 2015a *ILV* Brown, 2015a	New data Renewal

*acoording to SANCO 825/00 rev.8.1- Commodities and matrix groups:
1) dry commodities (high protein/high starch content);
2) commodities with high water content;
3) high oil content;
4) high acid content commodities.

For the purpose of renewal, the new validated multiresidue methods based on QuEChERS adapted procedure have been available. The methods were fully and independently validated and therefore can be recommended for the enforcement/monitoring purposes for trinexapac acid determination in food/feed of plant and animal origin with the LOQ of 0.01 mg/kg. For residue definition in food/feed of plant and animal origin for the enforcement please refer to 2.13 below.

Methods for trinexapac ethyl residue determination in soil, water, air

The summary of the new methods superceding the previous methods for trinexapac-ethyl residues determination in the environmental compartments provided in the table below.

Methods for trinexapac ethyl residue determination in the environmental matrices

Matrix	Analyte	Method principle ***LOQ***	Reference	EU review
soil (loamy silt) (sandy loam)	trinexapac ethyl (CGA163935)	HPLC-MS/MS monitoring two mass transitions *0.01 mg/kg*	GRM020.03A Hargreaves, 2008a *validation* Solé, 2008a	New data Renewal
soil (loamy silt) (sandy loam)	trinexapac (CGA179500) monitoring two mass transitions	HPLC-MS/MS monitoring two mass transitions *0.01 mg/kg*	GRM020.04A Hargreaves, 2008b *validation* Solé, 2008a	New data Renewal
soil (Loamy sand, LUFA 2.2) (Sandy loam, LUFA 5M)	CGA300405	HPLC-MS/MS monitoring two mass transitions *0.01 mg/kg*	GRM020.10A Braid, 2015 *validation* Heinz, 2015	New data Renewal
ground water drinking water	trinexapac ethyl (CGA163935) trinexapac (CGA179500)	HPLC-MS/MS *0.05 µg/L* *0.05 µg/L*	GRM020.02A Hargreaves, 2008c *validation* Solé, 2007	New data Renewal

surface water drinking water		monitoring two mass transitions	*ILV* Foster and Mumford, 2016	
surface water drinking water	CGA300405	HPLC-MS/MS *0.05 µg/L* monitoring two mass transitions	GRM020.11A Crook, 2015 *validation* Heinz, 2015a	New data Renewal
			ILV Hamberger, 2015	
air	trinexapac ethyl (CGA163935)	HPLC-MS/MS monitoring two mass transitions *10 µg/m³*	GRM020.12A Wiltshire K, 2015 *validation* Wiltshire K, 2015	New data Renewal

For the purpose of trinexapac-ethyl renewal the new fully validated LC-MS/MS methods with two mass transitions validated per analyte in the environmental samples – water, soil, air have been available. The methods are currently recommendable for the enforcement purposes to determine trinexapac, trinexapac-ethyl and metabolite CGA300405 (3-ethoxycarbonyl-pentanedioic acid) in the environment with the limits of quantification (LOQ) 0.01 mg/kg for soil, 0.05 µg/L for drinking and surface water and with the LOQ[1] of 10 $\mu g/m^3$ for air. For residue definition in the environmental matrices please refer 2.13 below.

[1] Acceptable the LOQ is below the concentration calculated from the $AOEL_{systemic}$ (10 µg/m3<102 $\mu g/m^3$)

2.6 Effects on human and animal health

It should be noted that the active substance trinexapac-ethyl is also referred as CGA163935 in the text of the document as this code is given by the notifier. Additionally, the main trinexapac-ethyl metabolite (4-[cyclopropyl(hydroxy)methylidene]-3,5-dioxocyclohexane-1-carboxylic acid, other IUPAC names: trinexapac and 4-(cyclopropyl-hydroxy-methylene)-3,5-dioxo-cyclohexanecarboxylic acid; CAS No 143294-89-7) is referred as CGA179500 in the text of the document as this code is given by the notifier.

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals

Table 17: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Test substance: [^{14}C-UL-Cyclohexyl]-CGA163935, batch GAN-XVI-38 (low doses), chemical purity and appearance not indicated, s.a. 30 µCi/mg, radiochemical purity 98.2%. [^{14}C-UL-Cyclohexyl]-CGA163935, batch CL-XVIII-31 (high dose), chemical purity and appearance not indicated, s.a. 1.0 µCi/mg, radiochemical purity 98.0%. CGA163935, Code S87-1209, chemical purity 96.6%, appearance not indicated ADME according to US EPA Guideline No. 85-1 "General Metabolism – rat"; make no reference to but partly in accordance with OECD 417 (1984) GLP Route/Dose (average mg/kg bw): Single intravenous low dose 0.91 Single oral low dose 0.97 Single oral high dose 166 repeated oral dose (14 days unlabelled + 1day 14C-labelled) : 1 (unlabelled), 0.97 (labelled) Investigations: Radioactivity distribution in excreta and tissues, metabolites in excreta Species: rat, CD albino; Group size: 5/sex/dose	Oral absorption, 168 h after administration, was 96-98%, based on radiolabel recovered from urine, cage wash, carcass and tissues. >94% of the dose was excreted in urine and faeces over 48 h. In most tissues, radioactive residues were below the limit of detection, 168 h after single or repeated oral dosing. Trinexapac-ethyl was eliminated almost completely (91-96% of dose) in the urine in the form of a single metabolite CGA179500 24 hours after oral dose.	The study is considered acceptable.	Anonymous, 1990 B.6.1.1 Study 1
Test substance: [1,2,6-^{14}C-cyclohexyl]-CGA163935, batch GAN-XVII-72, purity >96%, colourless crystals, s.a. 50 µCi/mg, radiochemical purity not indicated. CGA163935, batch AMS 265/101, purity 99.3%, colourless crystals ADME partly in accordance with OECD 417 (1984) GLP The bile duct cannulation experiment Route/Dose: oral single low (1.0-1.1 mg/kg bw) and high (198-207 mg/kg bw) dose	The blood kinetic values indicate that no apparent saturation of absorption was observable. Low dose- Cmax 0.5-1.3 ppm, Tmax 0.25 h, Blood T1/2 <0.6 h; AUC 0.25-48h 1 µg h equiv/g; tissues slow phase T1/2 ≤3.2 h High dose- Cmax 73-85 ppm, Tmax 0.25 h, Blood T1/2 <0.8 h; AUC 0.25-48h 170 µg h equiv/g; tissues slow phase T1/2 ≤12 h ≥82% of the administered radiolabel was absorbed, based on radiolabel	There are some discrepancies in results between the two oral toxicokinetic studies. When in doubt, the benefit of it is given to the first study with intact animals reported by Anonymous (1990) B.6.1.1 Study 1.	Anonymous, 1995 B.6.1.1 Study 2

Method	Results	Remarks	Reference
Investigations: blood kinetics, tissue distribution, bile excretion and metabolism Species: rat, Tif:RAI f (SPF) Group size: 3-4/sex/dose/time point	recovered from urine, cage wash and residual carcass, 48 h after single oral low dose administration. 79% of the administered radiolabelled dose was excreted in urine. The radiolabel recovered from urine after single oral low dose administration consisted for 92% of CGA179500, the other 8% represented an unidentified metabolite, which was the main metabolite discovered in bile (94% of the biliary radiolabel).		
Test substance: Trinexapac-ethyl, chemical purity 96.6%, Radiolabelled [cyclohexyl-1-2-6-^{14}C]-trinexapac-ethyl; Radiochemical purity: 99.7 % GLP The study is considered to be acceptable	All the human metabolites formed were detected in rat. In rat and human liver microsomes, metabolite M2 accounted for a mean of 97.1% and 40.5% of the sample radioactivity, following 60 minutes incubation, respectively.	No specific testing regulations or guidelines applicable for such study	Anonymous , 2017 B.6.1.3

2.6.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The toxicokinetic of trinexapac-ethyl was investigated in two studies using radiolabelled test substance in rats administered doses of ca. 1 mg/kg bw and ca. 200 mg/kg bw. The following test substances were used in the first study (Anonymous, 1990 (B.6.1.1 Study 1): [^{14}C-UL-Cyclohexyl]-CGA163935, batch GAN-XVI-38 (low doses), s.a. 30 µCi/mg, radiochemical purity 98.2%; [^{14}C-UL-Cyclohexyl]-CGA163935, batch CL-XVIII-31 (high dose), s.a. 1.0 µCi/mg, radiochemical purity 98.0%; CGA163935, Code S87-1209, chemical purity 96.6%. The following test substances were used in the second study (Anonymous, 1995 (B.6.1.1 Study 2): [1,2,6-^{14}C-cyclohexyl]-CGA163935, batch GAN-XVII-72, purity >96%, colourless crystals, s.a. 50 µCi/mg, radiochemical purity not indicated; CGA163935, batch AMS 265/101, purity 99.3%, colourless crystals.

There were some discrepancies between the two oral toxicokinetic studies reported. In the study with intact animals reported by Anonymous (1990) (B.6.1.1 Study 1) excretion of radiolabel in urine was ca. 91%, 24 h after application, while in the bile-cannulation experiment reported by Anonymous (1995) (B.6.1.1 Study 2) only ca. 79% had been excreted in urine, 48 h after administration. In the latter study also a high percentage of radiolabel was recovered from the gastro-intestinal tract (ca. 11% of the administered radiolabel). A high degree of inter-animal variability in data from urine and gastro-intestinal tract was observed. These discrepancies may reflect normal variability in results between laboratories and rat strains or may be due to the consequences of the surgical intervention applied to the rats in the study reported by Anonymous (1995) (B.6.1.1 Study 2). Because of the latter consideration, when in doubt, the benefit of it is given to the study with intact animals reported by Anonymous (1990) (B.6.1.1 Study 1).

Absorption

Absorption of trinexapac-ethyl (also referred as CGA163935) after oral administration to rats was at least 96%, irrespective of dose and sex, based on radiolabel recovered from urine, cage wash, carcass and tissues 168 after administration. Oral absorption of trinexapac-ethyl, 24 h after administration, was 91-94%, based on urine, irrespective of dose regimen or sex. The test substance was rapidly absorbed into the systemic circulation: independent of the sex and dose, maximum concentrations in blood occurred 15 min after oral administration.

Excretion

In rats, at least 95% of the administered radioactivity was eliminated in urine and 0.9-2.4% in faeces, 168 h after oral administration, irrespective of dose regimen or sex. More than 92% of the administered dose was excreted in the urine and faeces in the first 24 h. In male rats, a small part of the administered low dose was excreted in bile (ca. 3% in 48 h). Independent of the sex and dose, a half-life value ($t_{1/2}$) of blood levels was below 1 hour. Rapid phase elimination half times were similar for most tissues investigated (ca. 0.2 h after single oral low dose and ca. 0.7 h after single oral high dose). Slow phase elimination half times showed greater variability. Bone and liver showed the longest slow phase half times, both after low (3.2 and 2.3 h, respectively) and high dose administration (12 and 9.3 h, respectively).

Distribution

In male and female rats, 168 h after single or repeated oral dosing, radioactive residues in most tissues were below the limit of detection. Only in fat (low and high oral dose) and kidneys (high oral dose) a barely measurable but consistent level of radioactivity was found around the limit of detection: ca. 0.0015 mg eq/kg in the low dose groups (i.v. and oral) and 0.024 mg eq/kg in the high dose group for fat, and 0.017 mg eq/kg for the kidneys. The route of administration and the dose regimen had no influence on the residue levels. In a tissue distribution experiment with male rats after single oral administration, the highest concentrations of radiolabel were reached in kidneys, liver and plasma (at t_{max} (15 min) respectively 7.2, 3.0 and 1.5 mg eq/kg bw at low dose, and 553, 275 and 148 mg eq/kg bw at high dose). Bone showed the longest slow phase half-life ($T_{1/2}$), after low and high dose administration: 3.2 h and 12 h, respectively.

Metabolism

In rats, the major metabolite of trinexapac-ethyl (CGA163935) after oral administration was its free acid metabolite (referred as CGA179500), both in urine and faeces. Twenty-four hours after oral administration, ca. 22% and ca. 50% of the cumulative radiolabel recovered after single low dose from the faecal fraction consisted of parent compound and metabolite CGA179500, respectively. After repeated low dose and single high dose these values were, respectively, ca. 13 and ca. 39% as well as ca. 5% and ca.79%. Forty-eight hours after low dose administration, 92% of the cumulative urinary radiolabel consisted of this metabolite. The major biliary metabolite (94% of the biliary radiolabel) was not conclusively identified, the same metabolite was observed in urine (8% of the urinary radiolabel). It was probably a conjugate of either trinexapac-ethyl or CGA179500. No indication for saturation of metabolism was found after repeated low or single high dose oral administration.

In response to comment at renewal, the applicant has submitted a comparative *in vitro* rat and human metabolism study only (*Anonymous., 2017* (B.6.1.3)). The purpose of this study was to investigate the *in vitro* metabolism of [cyclohexyl-1-2-6-^{14}C]-trinexapac-ethyl ([^{14}C]-TXP) by rat and human liver microsomes and to compare the *in vitro* metabolite pattern in the rat and human test systems. Incubation samples were analysed by HPLC (0 and 60 minutes only) with radioactive monitoring and the proportions of the metabolites produced and parent [^{14}C]-TXP were quantified. Only 2 metabolite fractions (designated M1 and M2) were quantified common to Han Wistar rat and human liver microsomes. In the rat (male and female) and human (mixed gender) liver microsomal samples the mean [14C]-TXP was relatively stable at 0 min with 96.2% (rat) and 96.9% (human) of parent remaining. After incubation at 37°C for 60 minutes, the metabolism was quantitatively more extensive in rat liver microsomes with <1% of [14C]-TXP remaining, whereas, in human liver microsomes, metabolism was slower with 57.2% of parent remaining. Only metabolite fraction M2 was detected above the limit of quantification (≥ 1%) in rat and human liver microsomes after 60 minutes. In rat and human liver microsomes, metabolite M2 accounted for a mean of 97.1% and 40.5% of the sample radioactivity, following 60 minutes incubation, respectively.

2.6.2 Summary of acute toxicity

2.6.2.1 Acute toxicity - oral route

Table 18: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (Batch No; purity)	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 401 (1987) GLP The study is considered acceptable.	Rat: Tif: RAIf (SPF) hybrids of RII/1 x RII/2 5/sex/dose	Trinexapac-ethyl, P.705002, 96.6%	Doses: 2000, 5000 mg/kg bw Exposure: once by gavage	LD_{50}: >2000 and <5000 mg/kg bw	Anonymous, 1987b B.6.2.1 Study 1
OECD 401 (1987) Limit test GLP The study is considered acceptable.	Mouse: Tif: MAG f (SPF) 5/sex/dose	Trinexapac-ethyl, P.001010, 94.5%	Dose: 2000 mg/kg bw Exposure: once by gavage	LD_{50}: >2000 mg/kg bw	Anonymous, 1993 B.6.2.1 Study 2
OECD 401 (1987) GLP The study is considered acceptable.	Rat: Harlan Sprague Dawley 5/sex/dose	Trinexapac-ethyl, FL 881224, 96.9%	Doses: females: 3500, 4000, 5050 mg/kg bw males: 4000, 4500, 5050 mg/kg bw	LD_{50}: = 4210 mg/kg bw (female) LD50: = 4610 mg/kg bw (male) LD50: = 4460 mg/kg bw (sexes combined)	Anonymous, 1988 B.6.2.1 Study 3

Table 19: Summary table of human data on acute oral toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 20: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

(Q)SAR analysis using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited)	Trinexapac-ethyl (CGA 163935)	The results of (Q)SAR analysis) on parent substance were submitted (16-03-2017) on the request by the RMS. The software compares the structures with known toxicity endpoints. When a structural alert is identified the programme assigns a probability to the expression of toxicity by the compound.	Trinexapac-ethyl did not trigger Derek Nexus alert for 'High acute toxicity' endpoint. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.	Anonymous, 2017*

** - Syngenta has requested data confidentiality for these data pursuant to art.63 of Reg. (EC) 1107/2009*

2.6.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Three acute oral studies with trinexapac-ethyl are available. In two experiments the test species was the rat and in one experiment the test species was the mouse.

The acute oral LD_{50} of CGA 163935 tech. was found to be > 2000 and <5000 mg/kg bw for rats of both sexes (Anonymous, 1987b (B.6.2.1 Study 1)). Symptoms of toxicity included ruffled fur, dyspnoea, hunched posture and exophthalmos at a slight to moderate level in all groups. 3/5 males and 3/5 females administered 5000 mg/kg bw died in the period 2-4 days after exposure.

The acute oral LD_{50} of CGA 163935 tech. was found to be >2000 mg/kg bw for mice of both sexes (Anonymous, 1993 (B.6.2.1 Study 2)). Symptoms of toxicity (piloerection, hunched posture and dyspnoea) were observed in all animals. The severity of these effects gradually decreased and the animals had recovered completely by day 6 after administration. No deaths occurred during the observation period following administration of a limit dose of 2000 mg/kg bw.

The acute oral LD_{50} of the test substance was calculated to be 4610 mg/kg bw (95% confidence interval: 4450-4790 mg/kg bw) for male rats, 4210 mg/kg bw (95% confidence interval: 3450-5140 mg/kg bw) for female rats and 4460 mg/kg bw (95% confidence interval: 4180-4750 mg/kg bw) for the sexes combined (Anonymous, 1988 (B.6.2.1 Study 3)). Several animals in most dose groups had one or more of the following symptoms of toxicity: diarrhoea, nasal discharge, polyuria, salivation, decreased activity, piloerection, ataxia, dilated pupils, haematuria, and epistaxis. Discolouration of the contents of the gastrointestinal tract was observed in all the animals that died. A female dosed with 5050 mg/kg bw and one dosed with 4000 mg/kg bw had mottled red lungs.

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

Classification for acute oral toxicity under Regulation (EC) No 1272/2008 (Section 3.1) is required for substances with an acute oral LD_{50} value (or estimated LD_{50} value) of ≤2000 mg/kg bw. The lowest acute oral LD_{50} was 4210 mg/kg bw for female rats as reported by Anonymous (1988) (B.6.2.1 Study 3). Since the oral studies in rats and mice consistently revealed LD_{50} values >2000 mg/kg bw, classification for acute oral toxicity according to CLP regulation is not required.

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the available data, no classification is required for acute oral toxicity according to Regulation (EC) No 1272/2008.

2.6.2.2 Acute toxicity - dermal route

Table 21: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (Batch No; purity)	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 402 (1987) Limit test GLP The study is considered acceptable.	Rat: Tif: RAIf (SPF) hybrids of RII/1 x RII/2 5/sex/dose	Trinexapac-ethyl, P.705002, 96.6%	Dose: 4000 mg/kg bw on at least 10% of the body surface Exposure: 24 hours (semi-occlusive)	LD_{50}: >4000 mg/kg bw	Anonymous, 1987a B.6.2.2

Table 22: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 23: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
(Q)SAR analysis using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited)	Trinexapac-ethyl (CGA 163935)	The results of (Q)SAR analysis) on parent substance were submitted (16-03-2017) on the request by the RMS. The software compares the structures with known toxicity endpoints. When a structural alert is identified the programme assigns a probability to the expression of toxicity by the compound.	Trinexapac-ethyl did not trigger Derek Nexus alert for 'High acute toxicity' endpoint. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.	Anonymous, 2017*

** - Syngenta has requested data confidentiality for these data pursuant to art.63 of Reg. (EC) 1107/2009*

2.6.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

No deaths occurred in the acute dermal toxicity study at the limit dose of 4000 mg/kg bw (Anonymous, 1987a (B.6.2.2)). Signs of toxicity (including ruffled fur, dyspnoea, hunched posture, reduced spontaneous activity) were observed in all animals and persisted for up to nine days. No effects on body weight were observed. Gross necropsy did not reveal any treatment-related findings. The acute dermal LD_{50} of trinexapac-ethyl in the rat was therefore found to be >4000 mg/kg bw under the conditions of this study.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

Classification for acute dermal toxicity under Regulation (EC) No 1272/2008 (Section 3.1) is required for substances with an acute dermal LD_{50} value of ≤2000 mg/kg bw. Trinexapac-ethyl is reported to have an acute dermal LD_{50} of >4000 mg/kg bw; therefore classification is not required for acute dermal toxicity.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the available data, no classification is required for acute dermal toxicity according to Regulation (EC) No 1272/2008.

2.6.2.3 Acute toxicity - inhalation route

Table 24: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (Batch No; purity), form and particle size (MMAD(±gsd))	Dose levels, duration of exposure	Value LC_{50}	Reference
OECD 403 (1981) Limit test GLP The study is considered acceptable.	Rat: Tif: RAIf (SPF) hybrids of RII/1 x RII/2 5/sex/dose	Trinexapac-ethyl, P.705002, 96.6%; liquid 2.1 µm (±2.7 µm)	Dose: 5.3 ± 0.064 mg/L Exposure: 4 hours (nose only)	LC_{50}: >5.3 mg/L	Anonymous, 1988 B.6.2.3

Table 25: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 26: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

(Q)SAR analysis using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited)	Trinexapac-ethyl (CGA 163935)	The results of (Q)SAR analysis) on parent substance were submitted (16-03-2017) on the request by the RMS. The software compares the structures with known toxicity endpoints. When a structural alert is identified the programme assigns a probability to the expression of toxicity by the compound.	Trinexapac-ethyl did not trigger Derek Nexus alert for 'High acute toxicity' endpoint. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.	Anonymous, 2017*

** - Syngenta has requested data confidentiality for these data pursuant to art.63 of Reg. (EC) 1107/2009*

2.6.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

In the acute inhalation toxicity study (Anonymous, 1988 (B.6.2.3)), no deaths occurred. Signs of toxicity (ruffled fur, dyspnoea, hunched posture, and reduced spontaneous activity) were observed in all animals, including the control group, the first 6 hours. The effects persisted in the exposed rats up until day 7 of the observation period. No effects on body weight were observed. Gross necropsy did not reveal any treatment-related findings. The acute inhalation LC_{50} of trinexapac-ethyl in the rat was found to be >5.3 mg/L for rats of both sexes under the conditions of this study.

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

Classification for acute inhalation toxicity under Regulation (EC) No 1272/2008 (Section 3.1 of Annex I) is required for substances (dusts and mists) with an acute inhalation LC_{50} value of ≤5 mg/L. Trinexapac-ethyl is reported to have an acute inhalation LC_{50} of >5.3 mg/L; therefore classification is not required for acute inhalation toxicity.

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the available data, no classification is required for acute inhalation toxicity according to Regulation (EC) No 1272/2008.

2.6.2.4 Skin corrosion/irritation

Table 27: Summary table of animal studies on skin corrosion/irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance (Batch No; purity)</th><th>Dose levels, duration of exposure</th><th>Results
- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr><td>OECD 404 (1981)
GLP
Deviations: the application area was about three times larger than the area dictated by the guidelines (i.e. 20</td><td>Rabbit: New Zealand White
3 males</td><td>Trinexapac-ethyl,
P.705002, 96.6%</td><td>Dose: 0,5 ml on a skin area 20 cm^2
Exposure: 4 hours (occlusive)</td><td><table><tr><th>Scores observed after</th><th>1 hour</th><th>24 hours</th><th>48 hours</th><th>72 hours</th></tr><tr><td>Erythema</td><td>1, 1, 1</td><td>0, 0, 0,</td><td>0, 0, 0,</td><td>0, 0, 0,</td></tr><tr><td>Oedema</td><td>0, 0, 0,</td><td>0, 0, 0,</td><td>0, 0, 0,</td><td>0, 0, 0,</td></tr></table></td><td>Anonymous, 1987a
B.6.2.4</td></tr>
</table>

cm^2 in present study, while the guidelines specify 6 cm^2). The study is considered to be of supporting information and as supplementary element of weight of evidence approach.					

Table 28: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 29: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance (Batch No; purity)	Relevant information about the study (as applicable)	Observations	Reference
Acute dermal toxicity OECD 402 (1987) Limit test	Trinexapac-ethyl, P.705002, 96.6%	Species used: rat, Tif: RAIf (SPF) hybrids of RII/1 x RII/2 Group size, sex: 5/sex/dose Dose: 4000 mg/kg bw on at least 10% of the body surface	No local signs of skin irritation were reported. LD_{50}: >4000 mg/kg bw	Anonymous, 1987a B.6.2.2
Skin sensitisation *(M & K test)* OECD 406 (1992)	Trinexapac-ethyl, P.306042, 96.8%	Species used: guinea pig, Dunkin-Hartley Group size, sex: 10 controls, 20 test animals (males only)	Topical induction with the undiluted test substance (2 ml, 12.5 cm^2, 48 hours) caused no significant effect (no to slight erythema) in the guinea pigs (10% SDS was used). Epidermal application of the undiluted CGA 163935 tech. (1 ml, 6.25 cm^2, 48 hours) did not produce any irritation in the screening study for epidermal induction CGA 163935 tech. is not sensitising to the skin	Anonymous, 2001 B.6.2.6 Study 1
US EPA pesticide assessment Guideline No. 82-2 “21-day dermal – rat, rabbit, or guinea pig”; in accordance with OECD 410 (1981)	Trinexapac-ethyl, FL 872026, 96.6%	Species used: rabbit, New Zealand White Group size, sex: 5/sex/dose Short-term dermal exposure: 22 days, 6 h/d, semi-occlusive (10% of the total body surface area, ~240 cm^2) Doses: 0, 10, 100, 1000 mg/kg bw/d	At a dose of 10 mg/kg bw/d, 6 h/d did not result any irritation. At the mid- and high doses (100 and 1000 mg/kg bw/d) possibly the test substance had slight skin irritating effects (erythema scores ≤2). NOAEL ≥ 1000 mg/kg bw/d, no systemic effects	Anonymous, 1989 B.6.3.3.1
(Q)SAR	Trinexapac-	The results of (Q)SAR	Trinexapac-ethyl did not trigger Derek	Anonymous,

Type of study/data	Test substance (Batch No; purity)	Relevant information about the study (as applicable)	Observations	Reference
analysis using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited)	ethyl (CGA 163935)	analysis) on parent substance were submitted (16-03-2017) on the request by the RMS. The software compares the structures with known toxicity endpoints. When a structural alert is identified the programme assigns a probability to the expression of toxicity by the compound.	Nexus alert for 'Skin irritation' endpoint. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.	2017*

* - *Syngenta has requested data confidentiality for these data pursuant to art.63 of Reg. (EC) 1107/2009*

2.6.2.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

The submitted rabbit skin irritation study (Anonymous, 1987a (B.6.2.2)) showed only transient signs of slight erythema at 1 hour only. No skin irritation reactions were observed in any animal at 24, 48 and 72 hours after removal of the test article. However, it should be noted that the application area used in the study (Anonymous, 1987a (B.6.2.2)) was about three times larger than the area dictated by the guidelines (i.e. 20 cm^2 in present study, while the guidelines specify 6 cm^2). If the test substance is applied on an area that exceeds the recommended size, the sensitivity of the test might be reduced due to the thinner layer of substance on the skin.

The study follows the OECD 404 (adopted 12 May, 1981). After the study was performed, the OECD Test Guideline 404 has been revised in 1992, 2002 and 2015. Furthermore: *New Guidance document on Integrated Approached to Testing and Assessment (IATA) for Skin Irritation/Corrosion (OECD GD No 203; 2014)* and several Test Guidelines on *in vitro* methods for skin corrosion/irritation have been published. The study and original assessment doesn't fulfil these current scientific knowledge/data requirements. While there is significant departure from an OECD 404 (1981), the study could be considered as supplementary element of weight of evidence approach according to OECD Guidance document No 203 (2014). The study (Anonymous, 1987a (B.6.2.2)) is considered to be of supporting information, i.e. it gives supporting evidence regarding the skin effect of the CGA 163935 tech.

Additionally, to the findings of Anonymous study (1987a (B.6.2.2); OECD 402), a lack of skin irritation potential findings are consistent with observations from the submitted skin sensitization study (OECD 406; M & K, Anonymous, *2001* (B.6.2.6 Study 1)). Topical induction with the undiluted test substance (2 ml, 12.5 cm^2, 48 hours) caused no significant effect (no to slight erythema) in the guinea pigs (10% SDS was used). Furthermore: epidermal application of the undiluted CGA 163935 tech. (1 ml, 6.25 cm^2, 48 hours) did not produce any irritation in the screening study for epidermal induction.

Dermal exposure of rabbits to CGA 163935 at a dose of 10 mg/kg bw/day, 6 h/d for 22 days (OECD 410; Anonymous, *1989* (B.6.3.3.1)) did not result any irritation, however at the mid- and high doses (100 and 1000 mg/kg bw/day) possibly the test substance had slight skin irritating effects (erythema scores $\leq$2).

On the other hand negative results from other *in vivo* dermal toxicity data for CGA 163935 tech. (i.e. OECD 402, OECD 406, OECD 410) cannot justify a non-classification according to OECD Guidance document No 203 (2014).

Some additional data on non-testing methods (i.e. (Q)SAR analysis using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited) on substance trinexapac-ethyl were submitted on the request by the RMS LT: trinexapac-ethyl did not trigger in any Derek Nexus structural alert for skin irritation (for more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.).

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

Skin irritation is defined as the production of reversible damage to the skin following the application of a test substance for up to 4 hours (Section 3.2.1.1 of Annex I of the CLP Regulation). Classification of a substance for skin irritation (Category 2) is required on the basis of an animal study showing a mean value of ≥2.3 - ≤4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from three consecutive days after the onset of skin reactions. Classification is also required for inflammation that persists to the end of the observation period (normally 14 days) in at least 2 animals, particularly taking into account findings such as alopecia, hyperkeratosis, hyperplasia, and scaling. Classification may also be required in some cases where there is pronounced variability of response among animals, with very definite positive effects related to exposure in a single animal but less than the criteria listed above.

Based on weight of evidence analysis according to OECD Guidance document No 203 (2014) trinexapac-ethyl does not meet the criteria for classification as a skin irritant. Due to significant departure from an OECD 404 (1981) the submitted rabbit skin irritation study (Anonymous, 1987a (B.6.2.2)) was considered only as supplementary element of weight of evidence approach according to OECD Guidance document No 203 (2014). Additionally, to the findings of Anonymous, study (1987a) (B.6.2.2; OECD 402), a lack of skin irritation potential findings were consistent with observations from the submitted skin sensitization study (OECD 406; M & K, Anonymous, *2001* (B.6.2.6 Study 1)) and short term dermal study on rabbits (OECD 410; Anonymous, *1989* (B.6.3.3.1)). In addition, based on (Q)SAR analysis (using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited) submitted, trinexapac-ethyl did not trigger in any Derek Nexus structural alert for skin irritation.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Based on the available data, trinexapac-ethyl does not meet the criteria for classification as a skin irritant according to Regulation (EC) No 1272/2008.

2.6.2.5 Serious eye damage/eye irritation

Table 30: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (Batch No; purity)	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
OECD 405 (1987)	Rabbit: New Zealand	Trinexapac-ethyl, P.705002, 96.6%	Dose: 0.1 ml Exposure: single instillation in	Conjunctival redness 1-1-0 were observed in 2/3 animals at 1 hour after application.	Anonymous, 1987b

GLP The study is considered acceptable.	White 3 males		conjunctival sac of the left eye The treated eyes were not washed after instillation of the test substance.	The test substance generated mean score of corneal opacity 0-0-0, iritis 0-0-0, and conjunctival redness 0-0-0 and of oedema (chemosis) 0-0-0 of 3 tested animals under the conditions tested at 24, 48 and 72 hours after installation of the test material. No abnormal findings were observed in the treated eye of animals up to 3 days after treatment.	B.6.2.5

Table 31: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 32: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
(Q)SAR analysis using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited)	Trinexapac-ethyl (CGA 163935)	The results of (Q)SAR analysis) on parent substance were submitted (16-03-2017) on the request by the RMS. The software compares the structures with known toxicity endpoints. When a structural alert is identified the programme assigns a probability to the expression of toxicity by the compound.	Trinexapac-ethyl did not trigger Derek Nexus alert for 'Eye irritation' endpoint. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.	Anonymous, 2017*

** - Syngenta has requested data confidentiality for these data pursuant to art.63 of Reg. (EC) 1107/2009*

2.6.2.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

One eye irritation study in the rabbit is available (Anonymous, 1987b (B.6.2.5)). 3 rabbits were exposed to the test article without eye washing and conjunctival redness were observed in 2/3 only 1 hour after application (individual scores were 1 for both). Whereas no other ocular changes were observed in any animal during three days observation period, trinexapac-ethyl does not require classification for serious eye damage (Category 1) or for eye irritation (Category 2) according to Regulation (EC) No 1272/2008.

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

Serious eye damage (Category 1) is defined as the production of tissue damage in the eye, or serious physical decay of vision, following application of a substance to the anterior surface of the eye, which is not fully reversible within 21 days of application (Section 3.3.1.1 of Annex I of the CLP Regulation).

Eye irritation (Category 2) is defined as the production of changes in the eye following the application of test substance to the anterior surface of the eye, which are fully reversible within 21 days of application (Section 3.3.1.1 of Annex I of the CLP Regulation).

Classification in Category 1 is required for substances producing (in at least in one animal) effects on the cornea, iris or conjunctivae that are not expected to reverse or have not fully reversed within the observation period normally 21 days. Classification is also required where (in at least 2 of 3 animals) mean scores of ≥3 for corneal opacity or >1.5 for iritis are attained following grading at 24, 48 and 72 hours after installation of the test material.

Classification in Category 2 is required for substances producing (in at least 2 of 3 animals) mean scores of ≥1 for corneal opacity, ≥1 for iritis, ≥2 for conjunctival redness (erythema) and/or ≥2 for oedema (chemosis) following grading at 24, 48 and 72 hours after installation of the test material.

In the single study available (Anonymous, 1987b (B.6.2.5)), findings were limited to conjunctival redness (mean score of 1) in 2/3 animals at 1 hour after application. Trinexapac-ethyl does therefore not require classification for serious eye damage (Category 1) or for eye irritation (Category 2) according to Regulation (EC) No 1272/2008.

2.6.2.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Trinexapac-ethyl is not classified for eye irritation according to Regulation (EC) No 1272/2008 on the basis of the available data.

2.6.2.6 Respiratory sensitisation

Table 33: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
No data are available					

Table 34: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 35: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
(Q)SAR analysis using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited)	Trinexapac-ethyl (CGA 163935)	The results of (Q)SAR analysis) on parent substance were submitted (16-03-2017) on the request by the RMS. The software compares the structures with known toxicity endpoints. When a structural alert is identified the programme assigns a probability to the expression of toxicity by the compound.	Trinexapac-ethyl did not trigger Derek Nexus alert for 'Respiratory sensitisation' and/or 'Occupational asthma' endpoints. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.	Anonymous, 2017*

** - Syngenta has requested data confidentiality for these data pursuant to art.63 of Reg. (EC) 1107/2009*

2.6.2.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No data are available on the potential of trinexapac-ethyl to cause respiratory sensitisation. In addition, based on (Q)SAR analysis (using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited) submitted, trinexapac-ethyl did not trigger in any Derek Nexus structural alert for respiratory sensitisation and/or occupational asthma. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

A respiratory sensitiser is described as a substance that will lead to hypersensitivity of the airways following inhalation of the substance (Section 3.4.1.1 of Annex I of the CLP Regulation). Respiratory sensitisers are allocated into Sub-category 1A (strong sensitisers) or Sub-category 1B (other sensitisers), based on a weight of evidence from reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals. Substances are classified as Category 1 respiratory sensitisers where data are not sufficient for sub-categorisation, if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity, and/or if there are positive results from an appropriate animal test. Substances are classified as Sub-category 1A respiratory sensitisers where there is evidence of a high frequency of occurrence in humans, or a probability of occurrence of a high sensitisation rate in humans based on animal or other tests. Substances are classified as Sub-category 1B respiratory sensitisers where there is evidence of a low to moderate frequency of occurrence in humans, or a probability of occurrence of a low to moderate sensitisation rate in humans based on animal or other tests.

In the absence of relevant human or non-human data, trinexapac-ethyl is not classified as a respiratory sensitiser.

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

In the absence of any data, trinexapac-ethyl does not require classification for respiratory sensitisation according to Regulation (EC) No 1272/2008.

2.6.2.7 Skin sensitisation

Table 36: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (Batch No; purity)	Dose levels duration of exposure	Results	Reference
OECD 406 (1992), GLP *Magnusson and Kligman Maximisation* test Deviations: one animal from a not-specified group had removed its bandage before completion of the 24 hour challenge exposure. The study is considered acceptable.	Guinea pig: Dunkin-Hartley 10 controls, 20 test animals (males only)	Trinexapac-ethyl, P.306042, 96.8%	**Induction:** 10% m/v intradermal; 100% (undiluted) topical and 10% SLS in vaseline; **Challenge:** 100% (undiluted) topical (occlusive, 48h) and 10% SLS in vaseline; Vehicle: arachis oil, and FCA for the intradermal induction **Range-finding study:** 0.5, 1, 2.5, 5, 7.5 and 10% m/v in arachis oil for intradermal injections. Only localised reactions at 10% m/v. No skin reactions observed at 12.5, 50 and 75% m/m in arachis oil plus the undiluted liquid for topical induction (FCA-treated animals) and for topical challenge.	**Non-sensitiser** **Test:** 4/20 animals had slight erythema and 1/20 animal has well-defined erythema (classified as a positive reaction) after 24 hours. The erythema had cleared completely within 48 hours. **Negative control:** 4/10 animals had slight erythema after 24 hours. **Positive control** (2-mercaptobenzothiazole) data confirmed the sensitivity of the test system	Anonymous, 2001 B.6.2.6. Study 1
OECD 429 (2002) LLNA Limitations: dermal irritation data were not considered in selecting the concentrations to maximise	Mouse: CBA/Ca/Ola/Hsd 4 mice/group	Trinexapac-ethyl SMO5D180, 96.6%	Tested concentrations: 5%, 10% and 25% w/v Vehicle: acetone / olive oil (4:1 v/v)	**Non-sensitiser** A stimulation index (SI) is less than 3.0 However, it seems like a higher concentration should have been tested in order to get a reliable result.	Anonymous, 2006 B.6.2.6 Study 2

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance (Batch No; purity)	Dose levels duration of exposure	Results	Reference
expose and therefore, not suitable / high enough concentrations were selected. The study acceptability and reliability is considered to be questionable					
OECD 429 (2010) LLNA The study is considered to be acceptable.	Mouse: CBA/J 4 mice/group	Trinexapac-ethyl SMO5D180_FORTIFIED, 93.3%	Tested concentrations: 25%, 50% and 100% w/v Vehicle: 1% Pluronic® L92	**Sensitiser** **Skin Sens. 1B, H317** A stimulation index (SI): 1.57, 1.23 and 3.18, respectively EC3 value 95.4%	Anonymous, 2017 B.6.2.6 Study 3

Table 37: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 38: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
(Q)SAR analysis using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited)	Trinexapac-ethyl (CGA 163935)	The results of (Q)SAR analysis) on parent substance were submitted (16-03-2017) on the request by the RMS. The software compares the structures with known toxicity endpoints. When a structural alert is identified the programme assigns a probability to the expression of toxicity by the compound.	Trinexapac-ethyl triggered PLAUSIBLE Derek Nexus alert for 'Skin sensitisation in mammal' endpoint due to the presence of a diketone moiety. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.	Anonymous, 2017*

** - Syngenta has requested data confidentiality for these data pursuant to art.63 of Reg. (EC) 1107/2009*

2.6.2.7.1 Short summary and overall relevance of the provided information on skin sensitisation

In a guideline compliant GLP dermal sensitisation study (Anonymous, 2001 (B.6.2.6. Study 1)), twenty Dunkin-Hartley strain male guinea pigs were tested using the Magnusson and Kligman test. Induction and challenge dose were based on a range-finding study. The highest concentration that produced no significant irritation by topical application was 100%. None of the concentrations used topically in the range-finding test caused slight irritation, and the animals in the main study were therefore pretreated with 10% SLS in vaseline to increase the skin sensitivity.

Intradermal injection of 10% m/v and topical induction with the undiluted test substance caused no significant effects in the test animals compared with the control animals. Following challenge with undiluted test material, 4/20 test animals and 4/10 control animals had slight erythema on the test site after 24 hours, while 1/20 animals has well-defined erythema (classified as a positive reaction). The erythema had cleared completely within 48 hrs.

Sensitisation of this strain of animals was positively tested with 2-mercaptobenzothiazole. On the basis of the results, it was concluded that trinexapac-ethyl had no skin sensitization potential under the conditions of this study.

Additionally, the notifier Syngenta has submitted two Local Lymph Node Assays (Anonymous, *2006* (B.6.2.6. Study 2); Anonymous, *2017* (B.6.2.6. Study 3)). Trinexapac-ethyl (Batch No SMO5D180, purity 96.6%) was negative in the first LLNA study (Anonymous, *2006* (B.6.2.6. Study 2)); however, not high enough concentration was tested. The study follows the OECD TG 429 (2002), with exception of some limitations: dermal irritation data were not considered in selecting the concentrations to maximise expose and therefore, not suitable / high enough concentrations were selected. Additionally, dermal irritation at site of administration for each animal was not reported. The tested concentrations (5%, 10% and 25% w/v), the test compound (trinexapac-ethyl, Batch No SMO5D180, 96.6%) was not found to be sensitised. Dose levels were determined by highest achievable concentration in the preferred LLNA vehicles (acetone / olive oil (4:1 v/v)). However, higher concentrations should have been tested in order to get a reliable result. In addition, possibly other vehicle and the neat test substance should have been applied to achieve higher concentration of the test compound. Consequently, acceptability and reliability of this study was considered to be questionable.

In the other reliable study (Anonymous, *2017* (B.6.2.6. Study 3)) trinexapac-ethyl tech. (Bach No SMO5D180 Fortified, purity 93.3%) was considered to be a contact dermal sensitiser. This technical material was spiked with several impurities up to the maximum level they are proposed for inclusion in the technical specification proposed by the notifier and a comparison of the technical specification proposed by the RMS LT with the specification of the material used in this study is presented in the confidential part (RAR Volume 4CA Syngenta, points C.1.4.1. and C.1.4.2.). Concentrations tested (25%, 50% and 100% w/v) were selected based on toxicity, solubility, irritancy, and viscosity. As a stimulation index (SI) of greater than 3.0 was observed in one of the treatment groups (the neat test substance (100%)), the test substance was considered positive for a dermal sensitisation potential. No dermal irritation was observed for any of the vehicle (1% Pluronic® L92) and test sites. The EC3 value calculated for the test substance was 95.4%. This indicates that the test substance has moderate skin sensitisation potency. Proper conduct of the LLNA was confirmed via a positive response (SI = 3.44) with 25% HCA (alpha-Hexylcinnamaldehyde in 1% Pluronic® L92), a moderate contact sensitiser. Based on the estimated concentration three (EC3 value > 2%) it was concluded that trinexapac-ethyl (fortified) fulfilled the criteria for classification Skin Sens. 1B, H317 under the conditions of this study.

It should be noted that, however, based on (Q)SAR analysis (using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited) submitted, trinexapac-ethyl (CGA163935) triggered PLAUSIBLE Derek Nexus alert for skin sensitisation in mammal due to the presence of a diketone moiety. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

A skin sensitiser is defined as a substance that will lead to an allergic response following skin contact (Section 3.4.1.2 of Annex I of the CLP Regulation). Skin sensitisers are allocated into sub-category 1A (strong sensitisers) or sub-category 1B (other sensitisers), based on a weight of evidence of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals.

Substances are classified as Category 1 skin sensitisers where data are not sufficient for sub-categorisation, if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons, or if there are positive results from an appropriate animal test.

Substances are classified as sub-category 1A skin sensitisers where there is evidence of a high frequency of occurrence in humans and/or a high potency in animals. For LLNA, substances are allocated to sub-category 1A where EC3 value ≤2%. For the Guinea pig maximisation test, substances are allocated to sub-category 1A where a response of ≥30% is seen at intradermal induction concentrations of ≤0.1%; or where a response of ≥60% is seen at intradermal induction concentrations of >0.1% to ≤1%.

Substances are classified as sub-category 1B skin sensitisers where there is evidence of a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals. For LLNA, substances are allocated to sub-category 1B where EC3 value >2%. For the Guinea pig maximisation test, substances are allocated to sub-category 1B where a response of ≥30% to <60% is seen at intradermal induction concentrations of >0.1% to ≤1%; or where a response of ≥30% is seen at intradermal induction concentrations of >1%.

Substance trinexapac-ethyl (Bach No SMO5D180 Fortified, purity 93.3%) gave a positive result in the LLNA with an EC3 value of 95.4%. As this EC3 value is above the cut-off of 2%, the substance is considered to be a moderate skin sensitiser, and should be classified as a Category 1 (Sub-category 1B) skin sensitiser.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

Trinexapac-ethyl does warrant classification for skin sensitisation in accordance with Regulation (EC) No 1272/2008 on the basis of the available data.

2.6.2.8 Phototoxicity

Table 39: Summary table of studies on phototoxicity

Method, guideline, deviations if	Test substance (Batch No/ purity)	Dose levels duration of exposure	Results	Reference

any				
OECD 432 (2004) GLP Some limitations The study is considered acceptable.	Trinexapac-ethyl, SMO5D180, 96.6%	**Doses**: 1000; 316; 100; 31.6; 10; 3.16; 1.00 and 0.316 µg/mL, negative control (EBSS), blank (EBSS) and positive control (Chlorpromazine) **Exposure:** BALB/3T3 mouse fibroblast cell were treated for 1 h with different concentrations of the test solution and further 50 min in absence and in presence of a non-toxic dose of UVA light.	The highest test concentration of trinexapac-ethyl (1000 µg/mL) reduced viability of the cells to 81.7% (with irradiation). Viability in absence of UVA light was 102.7%. No EC_{50} values could be determined and a PIF could not be calculated. The mean phototoxic effect (MPE) was 0.010. This indicates no phototoxic potential (<0.1).	Gehrke, 2015

Table 40: Summary table of human data on phototoxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 41: Summary table of other studies relevant for phototoxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
(Q)SAR analysis using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited)	Trinexapac-ethyl (CGA 163935)	The results of (Q)SAR analysis) on parent substance were submitted (16-03-2017) on the request by the RMS. The software compares the structures with known toxicity endpoints. When a structural alert is identified the programme assigns a probability to the expression of toxicity by the compound.	Trinexapac-ethyl did not trigger Derek Nexus alert for 'Phototoxicity' endpoint. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.	Anonymous, 2017*

** - Syngenta has requested data confidentiality for these data pursuant to art.63 of Reg. (EC) 1107/2009*

2.6.2.9 Aspiration hazard

Table 42: Summary table of evidence for aspiration hazard

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data are available				

2.6.2.9.1 Short summary and overall relevance of the provided information on aspiration hazard

'Aspiration' is defined as the entry of a liquid or solid substance or mixture directly through the oral or nasal cavity, or indirectly from vomiting, into the trachea and lower respiratory system (Section 3.10.1.2 of Annex I of the CLP Regulation). Aspiration toxicity includes severe acute effects such as chemical pneumonia, varying degrees of pulmonary injury or death following aspiration. Substances are classified as hazard Category 1 for aspiration toxicity if they meet the following criteria: substances known to cause human aspiration toxicity hazards or to be regarded as if they cause human aspiration toxicity hazard; a classification is based on reliable and good quality human evidence or if it is a hydrocarbon and has a kinematic viscosity of 20,5 mm 2 /s or less, measured at 40^{o} C.

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

In the absence of any relevant human data and whereas trinexapac-ethyl is not hydrocarbon, trinexapac-ethyl is not classified as a respiratory sensitiser.

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

Whereas trinexapac-ethyl is not hydrocarbon and in the absence of any relevant human data, trinexapac-ethyl does not require classification for aspiration hazard according to Regulation (EC) No 1272/2008.

2.6.2.10 Specific target organ toxicity-single exposure (STOT SE)

Table 43: Summary table of animal studies on STOT SE (specific target organ toxicity-single exposure)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
OECD 401 (1987) GLP The study is considered acceptable. Rat: Tif: RAIf (SPF) hybrids of RII/1 x RII/2 5/sex/dose	Trinexapac-ethyl, P.705002, 96.6% Doses: 2000, 5000 mg/kg bw Exposure: once by gavage	LD_{50}: >2000 and <5000 mg/kg bw	Anonymous, 1987b B.6.2.1 Study 1
OECD 401 (1987) Limit test GLP The study is considered acceptable. Mouse: Tif: MAG f (SPF) 5/sex/dose	Trinexapac-ethyl, P.001010, 94.5% Dose: 2000 mg/kg bw Exposure: once by gavage	LD_{50}: >2000 mg/kg bw	Anonymous, 1993 B.6.2.1 Study 2

<table>
<tr><td>OECD 401 (1987)
GLP
The study is considered acceptable
Rat: Harlan Sprague Dawley
5/sex/dose</td><td>Trinexapac-ethyl,
FL 881224,
96.9%
Doses:
females: 3500, 4000, 5050 mg/kg bw
males: 4000, 4500, 5050 mg/kg bw</td><td>LD_{50}: = 4210 mg/kg bw (female)
LD_{50}: = 4610 mg/kg bw (male)
LD_{50}: = 4460 mg/kg bw (sexes combined)</td><td>Anonymous, 1988
B.6.2.1 Study 3</td></tr>
<tr><td>OECD 402 (1987)
Limit test
GLP
The study is considered acceptable.
Rat: Tif: RAIf (SPF) hybrids of RII/1 x RII/2
5/sex/dose</td><td>Trinexapac-ethyl, P.705002, 96.6%
Dose: 4000 mg/kg bw on at least 10% of the body surface
Exposure: 24 hours (semi-occlusive)</td><td>LD_{50}: >4000 mg/kg bw</td><td>Anonymous, 1987a
B.6.2.2</td></tr>
<tr><td>OECD 403 (1981)
Limit test
GLP
The study is considered acceptable.
Rat: Tif: RAIf (SPF) hybrids of RII/1 x RII/2
5/sex/dose</td><td>Trinexapac-ethyl,
P.705002, 96.6%;
liquid
2.1 µm (±2.7 µm)
Dose: 5.3 ± 0.064 mg/L
Exposure: 4 hours (nose only)</td><td>LC_{50}: >5.3 mg/L</td><td>Anonymous, 1988
B.6.2.3</td></tr>
<tr><td>OECD 404 (1981)
GLP
Deviations: the application area was about three times larger than the area dictated by the guidelines (i.e. 20 cm2 in present study, while the guidelines specify 6 cm2).
The study is considered to be of supporting information and as supplementary element of weight of evidence approach.
Rabbit: New Zealand White
3 males</td><td>Trinexapac-ethyl,
P.705002, 96.6%
Dose:
0,5 ml on a skin area 20 cm^2
Exposure:
4 hours (occlusive)</td><td>Results:<table><tr><th>Scores observed after</th><th>1 hour</th><th>24 hours</th><th>48 hours</th><th>7
ho</th></tr><tr><td>Erythema</td><td>1, 1, 1</td><td>0, 0, 0,</td><td>0, 0, 0,</td><td>0, (</td></tr><tr><td>Oedema</td><td>0, 0, 0,</td><td>0, 0, 0,</td><td>0, 0, 0,</td><td>0, (</td></tr></table></td><td>Anonymous, 1987a
B.6.2.4</td></tr>
<tr><td>OECD 405 (1987)
GLP
The study is considered acceptable
Rabbit: New Zealand White
3 males</td><td>Trinexapac-ethyl,
P.705002, 96.6%
Dose: 0.1 ml
Exposure: single instillation in conjunctival sac of the left eye
The treated eyes were not washed after instillation of the test substance.</td><td>Results:
Conjunctival redness 1-1-0 were observed in 2/3 animals at 1 hour after application.
The test substance generated mean score of corneal opacity 0-0-0, iritis 0-0-0, and conjunctival redness 0-0-0 and of oedema (chemosis) 0-0-0 of 3 tested animals under the conditions tested at 24, 48 and 72 hours after installation of the test material.
No abnormal findings were observed in the treated eye of animals up to 3 days after treatment.</td><td>Anonymous, 1987b
B.6.2.5</td></tr>
</table>

OECD 406 (1992), GLP *Magnusson and Kligman Maximisation* test Deviations: one animal from a not-specified group had removed its bandage before completion of the 24 hour challenge exposure. The study is considered acceptable. Guinea pig: Dunkin-Hartley 10 controls, 20 test animals (males only)	**Induction:** 10% m/v intradermal; 100% (undiluted) topical and 10% SLS in vaseline; **Challenge:** 100% (undiluted) topical (occlusive, 48h) and 10% SLS in vaseline; Vehicle: arachis oil, and FCA for the intradermal induction **Range-finding study:** 0.5, 1, 2.5, 5, 7.5 and 10% m/v in arachis oil for intradermal injections. Only localised reactions at 10% m/v. No skin reactions observed at 12.5, 50 and 75% m/m in arachis oil plus the undiluted liquid for topical induction (FCA-treated animals) and for topical challenge.	Results: **Test:** 4/20 animals had slight erythema and 1/20 animal has well-defined erythema (classified as a positive reaction) after 24 hours. The erythema had cleared completely within 48 hours. **Negative control:** 4/10 animals had slight erythema after 24 hours. **Positive control** (2-mercaptobenzothiazole) data confirmed the sensitivity of the test system	Anonymous, 2001 B.6.2.6. Study 1
OECD 429 (2002) LLNA Limitations: dermal irritation data were not considered in selecting the concentrations to maximise expose and therefore, not suitable / high enough concentrations were selected. The study acceptability and reliability is considered to be questionable Mouse: CBA/Ca/Ola/Hsd 4 mice/group	Trinexapac-ethyl SMO5D180, 96.6% Tested concentrations: 5%, 10% and 25% w/v Vehicle: acetone / olive oil (4:1 v/v)	Non-sensitiser A stimulation index (SI) is less than 3.0 However, it seems like a higher concentration should have been tested in order to get a reliable result.	Anonymous, 2006 B.6.2.6 Study 2
OECD 429 (2010) LLNA The study is considered to be acceptable Mouse: CBA/J 4 mice/group	Trinexapac-ethyl SMO5D180_FORTIFIED, 93.3% Tested concentrations: 25%, 50% and 100% w/v Vehicle: 1% Pluronic® L92	**Sensitiser** **Skin Sens. 1B, H317** A stimulation index (SI): 1.57, 1.23 and 3.18, respectively EC3 value 95.4%	Anonymous, 2017 B.6.2.6 Study 3
Acute neurotoxicity study OECD 424 (1997) GLP Rat, Crl:CD(SD) 10/sex/dose The study is considered acceptable.	Trinexapac-ethyl, SMO8E551, 95.8% 0, 500, 1000, 2000 mg/kg bw/d Single oral dose, gavage	Neurotoxicity NOAEL: ≥ 2000 mg/kg bw/d Neurotoxicity LOAEL: Not obtained. No signs of neurotoxicity observed at highest dose tested Systemic NOAEL: ≥ 2000 mg/kg bw/d Systemic LOAEL: Not obtained. Did not cause adverse effects at highest dose tested	Anonymous, 2012 B.6.7.1.1

Table 44: Summary table of human data on STOT SE (specific target organ toxicity-single exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 45: Summary table of other studies relevant for STOT SE (specific target organ toxicity-single exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other studies relevant for STOT SE are available				

2.6.2.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure (STOT SE)

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance of concern that leads to impaired function, both reversible and irreversible, immediate and/or delayed and not specifically addressed by other hazard classes.

The information gained from the five acute toxicity studies in rats and mice is provided in Table 43. There is no indication that trinexapac-ethyl causes toxicity to specific organs after a single exposure because non-lethal effects were confined to very high doses and were rather unspecific. This assessment is further supported by the acute neurotoxicity study in rats (please refer to section 2.6.7; Anonymous, 2012) in which no evidence of neurotoxicity or other toxicologically significant findings were observed at dose levels of 500, 1000, and 2000 mg/kg bw (for more detailed data please refer to RAR Volume 3, section B.6.7.1.1.).

No evidence of narcotic effects was obtained in any toxicological study. There are currently no validated animal tests that deal specifically with respiratory tract irritation, therefore this endpoint was not investigated directly and there is limited evidence available. However, no signs of respiratory irritation were observed in the acute inhalation study.

2.6.2.10.2 Comparison with the CLP criteria regarding STOT SE (specific target organ toxicity-single exposure)

Classification in STOT SE Category 1 is required for substances that have produced significant toxicity in humans or that, on the basis of studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following a single exposure. Substances are classified in Category 1 on the basis of reliable and good quality evidence from human cases, or observations from animal studies in which significant and/or severe effects of relevance to human health were produced at generally low exposure concentrations. Exposure levels relevant to classification in Category 1 are defined (Section 3.8.2.1.9.3 of Annex I of the CLP Regulation) as ≤300 mg/kg bw (oral route, rat); ≤1000 mg/kg bw (dermal route, rat) and ≤1 mg/L (inhalation route, rat, dust/mist/fume).

Classification in STOT SE Category 2 is required for substances showing significant toxic effects of relevance to humans, in studies in experimental animals and at generally moderate exposure levels. Exposure levels relevant to classification in Category 1 are defined (Section 3.8.2.1.9.3 of Annex I of the CLP Regulation) as 2 000 ≥ C > 300 mg/kg bw (oral route, rat); 2 000 ≥ C > 1 000mg/kg bw (dermal route, rat) and 5.0 ≥ C > 1.0 mg/L (inhalation route, rat, dust/mist/fume).

Classification in STOT-SE Category 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.

In the absence of human data and in the absence of any effects (clinical signs or pathology) considered to constitute significant or severe effects in the acute oral, dermal or inhalation toxicity studies, classification of trinexapac-ethyl in Category 1 or Category 2 for STOT SE is not required.

With regard to Category 3 for STOT SE, signs following inhalation exposure to trinexapac-ethyl were indicative of non-specific, general toxicity. As there was no evidence of specific toxic effects on a target organ or tissue, no signs of respiratory tract irritation or narcotic effects, no classification for specific target organ toxicity (single exposure) is proposed.

2.6.2.10.3 Conclusion on classification and labelling for STOT SE (specific target organ toxicity-single exposure)

Trinexapac-ethyl does not require classification for STOT SE (Category 1, 2 or 3) according to Regulation (EC) No 1272/2008, based on the available data.

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity)

The short term toxicity of trinexapac-ethyl was studied in two oral studies in rats (28-days and 13-weeks), two oral studies in dogs (13-weeks and 1-year) and via dermal route in a 22-day study in rabbits. The results of short term toxicity studies are summarised in Table 46. However, three supplementary reports with additional information regarding the trinexapac-ethyl 1 year dog study (and partly a 13-week dog study) were given for the renewal of approval of the active substance trinexapac-ethyl. In addition, fourth supplementary report was issued by the applicant in response to the RMS conclusion regarding the trinexapac-ethyl 1 year dog study. In response to comment at renewal, the applicant has submitted the pilot 7-week oral feeding study in dogs.
In addition, there are chronic toxicity / carcinogenicity study in rats, carcinogenicity study mice and reproductive toxicity, neurotoxicity as well as immunotoxicity studies.

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE)

Table 46: Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (Batch No; purity), route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference

Short-term oral toxicity according to a technical guidance Merkblatt Nr.33/D-1.3; make no reference to but partly in accordance with OECD 407 (1981) GLP Albino rat Tif: RAIf (SPF) hybrids of RII/1 x RII/2 10/sex/dose The study is considered acceptable.	Trinexapac-ethyl, LV 609024, 95% Doses: 0, 10, 100, 1000/2000 mg/kg bw/d 28-day oral, gavage	NOAEL = 100 mg/kg bw /d (M)* LOAEL = 1000/2000 mg/kg bw /d, based on ↑ water consumption (M, F); ↑absolute and relative liver (M, F) & kidney (M) weight; liver and kidney histopathology (M) **Not suitable to establish a proper NOAEL for females*	Anonymous, 1988 B.6.3.1.1
Short-term oral toxicity according to US EPA pesticide assessment Guideline No. 82-1 “90-day oral – two species, rodent and nonrodent”; make no reference to but partly in accordance with OECD 408 (1981) GLP Rat: Sprague-Dawley 15/sex/dose The study is considered acceptable.	Trinexapac-ethyl, FL 872026, 96.6% Doses: 0, 50, 500, 5000, 20000 ppm Equal to 0, 3, 34, 346, 1350 mg/kg bw/d for males and 0, 4, 38, 395, 1551 mg/kg bw/d for females 90-day oral, dietary	NOAEL = 34 mg/kg bw /d (M) LOAEL= 346 mg/kg bw /d, based on histopathological kidney effects (M) NOAEL = 395 mg/kg bw /d (F) LOAEL= 1551 mg/kg bw /d, based on ↓ food consumption, ↓ body weight gain (11.1%) (F)	Anonymous, 1989a B.6.3.2.1
Short-term oral toxicity Pilot study No OECD TG GLP Beagle dog 3/sex/group The study is considered acceptable	Trinexapac-ethyl, FL 872026, 96.6% Doses: 0, 500, 5000, 15000 and 15000 (1-3 d) →30000 (4-28 d) →50000 (29 d onwards) ppm Equal to 0, 22.2, 218.7 685.8, and 685.8→956.2→733.6 (~861) mg/kg bw/d for males and 0, 23.1, 214.3, 679.9 and 679.9→1373.3→ 964.7 (~1198) mg/kg bw/d for females 7-week oral, dietary	NOAEL = 679.9 mg/kg bw /d (M & F) LOAEL= 861 mg/kg bw /d, based on ↓ body weight (M & F), ↓ percent body weight gain (M & F), ↓food consumption (M & F), ↓ absolute and relative thymus weight & thymus atrophy (M & F) ↓bw (M & F): >10% at days 35-49) ↓FC (M: 30 - 79% days at 7, 14 and 28; F: >56% at days 35 - 49)	Anonymous, 1989 B.6.3.2.4

Short-term oral toxicity according to US EPA pesticide assessment Guideline No. 82-1 "90-day oral – two species, rodent and nonrodent"; make no reference to but are partly in accordance with OECD 409 (1981) GLP Beagle dog 4/sex/dose The study is considered acceptable.	Trinexapac-ethyl, FL 872026, 96.6% FL 882373, 96.2% FL 881224, 94.6% Doses: 0, 50, 1000, 15000, 30000 ppm; Equal to 0, 2, 35, 516, 930 mg/kg bw/d for males and 0, 1.9, 40, 582, 890 mg/kg bw/d for females 90-day oral, dietary	NOAEL = 516 mg/kg bw /d (M & F) LOAEL= 890 mg/kg bw /d, based on clinical signs (emaciation) (M), ↓ body weight (M & F), ↓ body weight gain (M & F), ↓food consumption (M & F), ↓ absolute and relative thymus weight (M) & thymus atrophy (M & F) ↓bw (M: 26.1%; F: 11.7%) ↓bw gain: (M: -18.3%; F: -6.1%) ↓FC (M, F)	Anonymous, (1989b) B.6.3.2.2
Short-term oral toxicity OECD 453 (1981), make no reference to but are partly in accordance with OECD 452 (1981) GLP Beagle dog 4/sex/group The study is considered acceptable.	Trinexapac-ethyl, FL 882373, 96.2% FL 892178, 96.2% FL 891417, 92.2% Doses: 0, 40, 1000, 10000, 20000 ppm; Equal to 0, 1.6, 31.6, 365.7, 726.7 mg/kg bw/d for males and 0, 1.4, 39.5, 357.1, 783.8 mg/kg bw/d for females 1 year oral, dietary	NOAEL = 31.6 mg/kg bw /d (M & F) LOAEL= 357.1 mg/kg bw /d, based on clinical signs (faeces mucoid/bloody, M & F), ↓terminal bw (M: 11.5%), haematological changes (↓RBC, ↓HCT, ↓HGB) (F), possible effect on the oestrus cycle & decreased absolute uterus weight, brain histopathology (vacuolation) (M & F)	Anonymous,, 1992 B.6.3.2.3 four supplementary studies: B.6.3.2.3.1; B.6.3.2.3.2; B.6.3.2.3.3; B.6.3.2.3.4
Short-term dermal toxicity US EPA pesticide assessment Guideline No. 82-2 "21-day dermal – rat, rabbit, or guinea pig"; make no reference to but in accordance with OECD 410 (1981) GLP Species: rabbit, New Zealand White Group size: 5/sex/dose The study is considered acceptable.	Trinexapac-ethyl, FL 872026, 96.6% 22 days dermal, 6 h/d, semi-occlusive (10% of the total body surface area, ~240 cm^2) Doses: 0, 10, 100, 1000 mg/kg bw/d	NOAEL ≥ 1000 mg/kg bw/d No systemic effects	Anonymous, 1989 B.6.3.3.1

Combined chronic toxicity /carcinogenicity study OECD 453 (1981) GLP Rat, Sprague-Dawley [Crl:VAF/Plus CD (SD) Br] Chronic (104 weeks): 20/sex/dose Carcinogenicity (104 weeks): 50/sex/dose Interim sacrifice (52-weeks): 10/sex/dose Interim recovery (52 + 4-weeks recovery): 10/sex/dose (control and 20000 ppm) The study is considered acceptable, despite some deviations.	Trinexapac-ethyl, FL 872026, 96.9% FL 881224, 96.9% FL 882373, 96.2% FL 892178, 96.2% FL 891417, 92.2% 0, 10, 100, 3000, 10000, 20000 ppm Equal to 0, 0.4, 3.9, 115.6, 392.7, 805.7 mg/kg bw/d for males and 0, 0.5, 4.9, 147.4, 494.0, 1054.0 mg/kg bw/d for females 52/104-week oral, dietary	Long-term NOAEL = 115.6 mg/kg bw /d (M & F) Long-term LOAEL= 392.7 mg/kg bw /d (M & F), based on interim renal histopathological effects (hyaline droplets) and bile duct hyperplasia in the liver (M), galactoceles in mammary skin (F) NOAEL for carcinogenicity ≥ 805.7 mg/kg bw /d (M & F)	Anonymous, 1992 B.6.5.1
Carcinogenicity study OECD 451 (1981) GLP Mouse, Crl:CD-1(ICR)Br 70/sex/dose The study is considered acceptable.	Trinexapac-ethyl, FL 872026, 96.9% FL 881224, 96.9% FL 882373, 96.2% 0, 7, 70, 1000, 3500, 7000 ppm Equal to 0, 0.91, 9.01, 130.81, 450.72, 911.77 mg/kg bw/d for males and 0, 1.08, 10.66, 154.08, 538.73, 1073.42 mg/kg bw/d for females 78-week oral, dietary	Long-term NOAEL ≥ 911.8 mg/kg bw /d (M & F) (highest dose tested) There were no adverse effects NOAEL for carcinogenicity ≥ 911.8 mg/kg bw /d (M & F) (highest dose tested) There were no tumour incidences	Anonymous, 1991 B.6.5.2
Two-generation reproduction toxicity study OECD 416 (1983) GLP Rat, Sprague-Dawley 30/sex/group The study is considered acceptable, despite some deviations	Trinexapac-ethyl, FL 882373, 96.2% FL 892178, 96.2% 0, 10, 1000, 10000, 20000 ppm Equal to 0, 0.7, 106.2, 662.9 and 1293.0 mg/kg bw/d (average of all values) Oral: diet Approximate number of dose weeks: F0 – 22-25; F1 – 20-23	**Parental** NOAEL: 106.2 mg/kg bw/d LOAEL: 662.9 mg/kg/d; ↓bw gain premating (F0 males Day 0-91: 9.6%; F1 males Day 0-84: 10.5%; F0 female Day 0-91: 14.8%); ↓FC premating (F1 males: average 5.9%) **Offspring** NOAEL: 662.9 mg/kg bw/d LOAEL: 1293.0 mg/kg bw/d: **Reproductive** NOAEL: ≥ 1293.0 mg/kg bw/d LOAEL: Not obtained.	Anonymous, 1991 B.6.6.1.1

Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP Rat, Sprague-Dawley, RAIf (SPF) hybrids of RII/1 × RII/2 24 females / dose group The study is considered acceptable	Trinexapac-ethyl, P.705002, 96.6% 0, 20, 200, 1000 mg/kg bw/d Days 6-15 of gestation, gavage	**Maternal:** NOEL: ≥ 1000 mg/kg bw/d LOAEL: Not obtained. Did not cause adverse effects at highest dose tested. **Developmental:** NOAEL: 200 mg/kg bw/d LOAEL: 1000 mg/kg bw/d ↑ litter incidence of asymmetrically shaped sternebrae	Anonymous, 1988 B.6.6.2.1
Developmental toxicity (teratogenicity) study OECD 414 GLP Rabbit, New Zealand White 16-17 females / dose group The study is considered acceptable, despite deviation	Trinexapac-ethyl, P.705002, 96.6% 0, 10, 60, 360 mg/kg bw/d Days 7-19 of pregnancy, gavage	**Maternal:** NOAEL: 60 mg/kg bw/d LOAEL: 360 mg/kg bw/d: ↑mortality (2/17): 1 animal on day 13 (6 days after dosing), second was killed on day 24 due to marked and continuing weight loss and was found to have haemorrhagic depressions in the stomach, retarded body weight gain to Day 15 **Developmental:** NOAEL: 60 mg/kg bw/d LOAEL: 360 mg/kg bw/d: ↑ post-implantation loss; ↓ number of live foetuses	Anonymous, 1990 B.6.6.2.2
Subchronic (13 week) dietary neurotoxicity study OECD 424 (1997) GLP Rat, Crl:CD(SD) 12/sex/dose The study is considered acceptable.	Trinexapac-ethyl, SMO8E551, 95.8% 0, 3750, 7500, 15000 ppm Equal to 0, 233, 463, 948 mg/kg bw/d for males and 0, 294, 588, 1171 mg/kg bw/d for females 13 weeks oral, dietary	Neurotoxicity NOAEL: ≥ 948 mg/kg bw/d Neurotoxicity LOAEL: Not obtained. No signs of neurotoxicity observed at highest dose tested. Systemic NOAEL: ≥ 948 mg/kg bw/d Systemic LOAEL: Not obtained. Did not cause adverse effects at highest dose tested.	Anonymous, 2012a B.6.7.1.2
28-Day immunotoxicity feeding study Immunotoxicity US EPA OPPTS 870.7800 (1998) GLP Mouse (female), B6C3F1 10/females/group and subsets AFC/NKC The study is considered acceptable.	Trinexapac-ethyl, SMO5D180, 96.6% 0, 500, 2000, 5000 ppm Equal to average 0, 160.2, 613.7, 1630.5 mg/kg bw/d 28-days oral, dietary	Immunotoxicity NOAEL: ≥ 1530.5 mg/kg bw/d. Immunotoxicity LOAEL: Not obtained. No signs of immunotoxicity (the humoral and innate immune response) observed at highest dose tested. Systemic NOAEL: ≥ 1530.5 mg/kg bw/d Systemic LOAEL: Not obtained. Did not cause adverse effects at highest dose tested.	Anonymous, 2011 B.6.8.2.1

↓- *decrease compared to control;* ↑- *increase compared to control.*

Table 47: Summary table of human data on repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 48: Summary table of other studies relevant for repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
(Q)SAR analysis using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited)	Trinexapac-ethyl (CGA 163935)	The results of (Q)SAR analysis) on parent substance were submitted (16-03-2017) on the request by the RMS. The software compares the structures with known toxicity endpoints. When a structural alert is identified the programme assigns a probability to the expression of toxicity by the compound.	Trinexapac-ethyl did not trigger Derek Nexus alert for any endpoint relevant for repeated dose toxicity STOT RE (e.g. Bladder disorders, Bone marrow toxicity, Bradycardia, Cardiotoxicity, Cumulative effect on white cell count and immunology, Hepatotoxicity, Kidney disorders, Kidney function-related toxicity, Methaemoglobinaemia, Nephrotoxicity, Ocular toxicity, Pulmonary toxicity, Splenotoxicity, Thyroid toxicity and/or Urolithiasis). For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.	Anonymous, 2017*

** - Syngenta has requested data confidentiality for these data pursuant to art.63 of Reg. (EC) 1107/2009*

2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity)

Specific target organ toxicity (repeated exposure) is defined in the CLP Regulation (Section 3.9.1.1 of Annex I) as specific, target organ toxicity arising from repeated exposure to a substance. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included in this definition. The adverse health effects relevant for STOT RE classification include consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism and these changes are relevant for human health. With respect to animal data, Annex 1, Section 3.9.2.5 of the CLP Regulation notes that the standard animal studies in rats or mice that provide this information are 28-day, 90-day or lifetime studies (up to 2 years) that include haematological, clinicochemical and detailed macroscopic and microscopic examination to enable the toxic effects on target tissues/organs to be identified. Data from repeat dose studies performed in other species may also be used, if available and other long-term exposure studies such as carcinogenicity, neurotoxicity or reproductive toxicity may also provide evidence of specific target organ toxicity that could be used in the assessment of STOT RE classification.

Classification with STOT- RE is triggered by the occurrence of *significant* (and/or *severe* for Category 1) toxic effects at doses below specified guidance values. For STOT-RE Category 1, the relevant guidance values for oral exposure are 10 mg/kg bw/day (rat 90-day study) and 30 mg/kg bw/day (rat 28-day study). For STOT-RE Category 2, the relevant guidance values for oral exposure are 100 mg/kg bw/day (rat 90-day study) and 300 mg/kg bw/day (rat 28-day study).

Short-term oral toxicity

In a **28-days oral toxicity study** (Anonymous, 1988 (B.6.3.1.1)), rats were administered up to 1000/2000 mg/kg bw/day trinexapac-ethyl. As the exposed females were not properly fasted before sacrifice, it was difficult to assess which effects were treatment-related, and thus not possible to set a NOAEL. The NOAEL for males was set at 100 mg/kg bw/day, based on increased water consumption, increased in absolute and relative liver and kidney weight as well as liver and kidney pathology: hepatocellular hypertrophy in centrilobular regions of the liver and PAS-positive droplets in the epithelia of the collecting ducts of the kidneys.

A **13-week oral toxicity study** was performed on rats administered up to 20000 ppm (1350 and 1551 mg/kg bw/day for males and females, respectively) of trinexapac-ethyl. (Anonymous, 1989a (B.6.3.2.1)). A decrease in cumulative body weight gain and food consumption for females was observed at 20000 ppm. A statistically significant and dose related increase in relative liver weight was observed in the male dose groups of 5000 (346 mg/kg bw/day) and 20000 ppm. Since no histopathological or others changes were reported, this finding was not considered to be adverse. Increased relative weight (at 20000 ppm) and histopathological effects (at 5000 and 20000 ppm) were evident in the kidneys of males, the same target organ as in the 28-day rat study. Statistically significant increases in the incidence of renal tubular changes, including scattered foci of tubular basophilia and cytoplasmic accumulations of hyaline droplets in cortical tubular epithelium were observed. However, other effects associated with chronic progressive nephropathy in male rats were not observed in this and in combined toxicity/carcinogenicity studies, e.g. cell necrosis, defoliation of tubular epithelium, the hyperplasia, linear mineralization and renal tumours. Therefore, these renal histopathological effects observed in male rats were considered relevant for humans. The NOAEL for males was set at 500 ppm (34 mg/kg bw/day).

The NOEL in **7-week oral feeding dog study** (Anonymous, 1989 (B.6.3.2.4)) was set at 15000 ppm, equal to 679.9 mg/kg bw/d for females, due to the observed decreased mean absolute body weight, mean percent body weight gain and food consumption throughout the dosing period in both sexes at the highest dose levels (approximately 861 mg/kg bw/d for males). In addition, thymic atrophy as well as reduction in absolute and relative thymus weights in all dogs at the highest dose level was considered as non-specific secondary response to the presence of overt general toxicity. Increased relative weight and histopathological effects were evident in the kidneys of 5 males at approximately 861 mg/kg bw/d. No microscopic abnormalities of the brain were observed.

90-days dog study. Oral exposure of dogs to trinexapac-ethyl at concentrations of 30000 ppm (930 and 890 mg/kg bw/day for males and females, respectively) for 13 weeks (Anonymous, 1989b (B.6.3.2.2)) resulted in decreased terminal body weight, body weight gain and food consumption throughout the dosing period in male and female dogs, and emaciation in several males. These findings were the justification for setting the NOAEL at 15000 ppm, equal to 516 mg/kg bw/day. Effects on thymus weight in males in combination with diffuse thymic atrophy in both sexes in the highest dose group were considered as non-specific secondary response to the presence of overt general toxicity. It is noteworthy that in one male of eight dogs at the high level (930 mg/kg bw/day) the cerebral vacuolation was reported and later confirmed by supplementary report (Krinke G., 1994 (B.6.3.2.3.3.)).

In a **52-wk oral toxicity study in dogs** (Anonymous, *1992* (B.6.3.2.3)) treatment-related clinical signs (mucoid/bloody faeces) occurred in both sexes at concentrations of ≥10000 ppm (365.7 and 357.1 mg/kg bw/day for males and females, respectively). Terminal bodyweights were non-statistically significantly lower compared to controls (9.8-11.5%) in the two high male and in the top female dose groups. A statistically significant decrease (>10%) in mean percent body weight gain throughout the dosing period occurred in males at ≥ 10000 ppm (365.7 mg/kg bw/day). At all doses, mean body weight gain (kg) at termination was reduced by 12.5-34.2% (no statistics performed) in males compared with the control group, however it was clearly affected in females at 20000 ppm only.

A treatment-related decrease (11.3 – 18.0%) in mean red blood cell count, haematocrit and in mean haemoglobin throughout the dosing period was seen in females at 10000 and 20000 ppm and was considered to be adverse. The reduction (>10%) in mean red blood cell count and haematocrit was statistically significant in male animals receiving 20000 ppm.

A statistically significant reduction in mean absolute and relative uterus weight (69-75%) occurred at concentrations ≥1000 ppm. Based on the supplementary report with additional information (*Krinke G., Mahrous A., 1999* (B.6.3.2.3.2.)), the reduction in mean absolute uterus weight at the two highest doses was a consequence of the physiological change occurring in the uterus at the late stages of the oestrus cycle: regression/inactivity of uterus glands and/or no glandular proliferation at these doses were established. No histopathological effects were seen in the uterus at any dose. Since a robust evaluation of oestrus cyclicity and hormone analysis was not carried out as well as a number of methodological deficiencies were identified in this specific supplementary report (the unclear origin of the classification scheme, only the histology of the uterus reported, the use of a single time point and the low number of animals), it was difficult to assess the biological relevance of the results. However, an adverse effect of trinexapac-ethyl on the oestrus cycle via a hormonally mediated mechanism at the two highest doses cannot be ruled out and therefore this effect was considered toxicologically relevant and the LOAEL for these findings was set at 10000 ppm (equal to 357.1 mg/kg bw/day for female).

A treatment-related and dose dependent vacuolation of forebrain and midbrain regions was seen at 10000 and 20000 ppm. The incidences were statistically significantly increased only at 20000 ppm. The compound-related vacuoles noted at 10000 ppm and 20000 ppm, although still small, were generally larger in size and more closely clumped than the artefactual vacuoles from control and other dogs. The two supplementary reports with additional information regarding effects of the trinexapac-ethyl on brain were given for the renewal of approval of the active substance (*Persohn E, 1999* (B.6.3.2.3.1.) and *Krinke G., 1994* (B.6.3.2.3.3.)). The topographical distribution of the lesion involved three forebrain and two midbrain regions at 20000 ppm as well as one forebrain region at 10000 ppm in both sexes. The vacuolation was mostly located in the white brain matter, in the zone of transition between the white and the grey brain matter. The lesion was confined to a bilateral - symmetrical swelling of oligodendroglial and astrocytic cells, without progression to more advanced or more extensive damage of the nervous tissue. Nerve cells were not vacuolated. The cerebral vacuolation was treatment-related and evident age-dependent as well as dosage-dependent by comparison of 7-week, 13-week and 52-week feeding studies. The dog was found to be most susceptible species with regard to the cerebral vacuolation effects as they were not observed in species other than dog. The observed cerebral vacuolation in dogs was neither the result of a myelinopathy nor astrogliosis/astrocytosis. The lesion was not inflammatory in character. The mild, probably reversible effect on glial cells was probably induced by an interference with glucose metabolism and/or synthesis of nucleic acids and proteins. However, whether the observed cerebral vacuolation in dogs had any relationship with adverse effects in humans remained uncertain. Therefore, in the absent of mechanistic studies and/or any human data, the cerebral vacuolation was considered as relevant for human.

The no observable effect level (NOEL) in the 1 year dog study was 1000 ppm (equal to 31.6 mg/kg bw/day for males) for both sexes based on adverse toxic effects the next higher dose group (10000 ppm): clinical signs (mucoid/bloody faeces) in males and females, decreased terminal body weight in males, haematological findings (decreased RBC, haematocrit, haemoglobin) in females, changes in oestrus cyclicity, decreased absolute uterus weight as well as microscopic evidence of brain histopathology (cerebral vacuolation) in both sexes.

Short-term dermal toxicity

No systemic effects were observed in any group of rabbits administered up to 1000 mg/kg bw/day trinexapac-ethyl in **22-days dermal toxicity study** (Anonymous, 1989 (B.6.3.3.1)). Local skin irritation was evident in all dose groups and the vehicle control group. It is very likely that the local effects were caused by the vehicle, ethanol, which is a skin irritating substance. However, as the severity and/or number of animals with skin effects was slightly higher in the mid- and high dose groups, trinexapac-ethyl probably also has slight skin irritating effects after repeated exposure. The NOAEL for systemic effects was set at ≥ 1000 mg/kg bw/day in both sexes.

Long term toxicity / carcinogenicity

The repeated dose toxicity of trinexapac-ethyl has also been investigated in guideline cancer bioassays in rats and mice. These studies are addressed in section 2.6.5 (for more detailed data please refer to RAR Volume 3, section B.6.5).

52/104-week combined chronic toxicity and carcinogenicity study in the rat. Here, it is sufficient to state that in the 104-wk combined chronic toxicity /carcinogenicity study in rats exposed to up to 20000 ppm (805.7 mg/kg bw/day for males and 1054.0 mg/kg bw/day for females), mortality was >50% in all dose groups except the male high dose group. Statistically significant reductions (>10%) in mean body weight, percent body weight gain and food consumption occurred intermittently in males and females at 20000 ppm throughout the study but not at study termination. Hence, there were no adverse effects on mean body weight, percent body weight change and food consumption at concentrations ≤10000 ppm (392.7 mg/kg bw/day for males and 494.0 mg/kg bw/day for females). The NOAEL for long-term effects was set at 3000 ppm (115.6 mg/kg bw/day for males and 147.4 mg/kg bw/day for females), based on an increase in the incidence of bile duct hyperplasia in the livers of males and galactoceles in mammary skin of females at the next higher dose level. In addition, following the initial 52 weeks of the study renal histopathological effects (hyaline droplets) were observed in 10000 ppm and 20000 ppm males.

78-week oral carcinogenicity study in mouse. It is sufficient to state that there were no clinical signs of toxicity and no treatment-related effects on survival, haematology, ophthalmology, organ weights or macroscopic findings in 78 weeks carcinogenicity study in mice. The observed effects on body weight, percent body weight gain and food consumption were not considered to be adverse. Under the conditions of this study, the maximal tolerated dose (MTD) seems not to be reached. Dietary administration of trinexapac-ethyl for 78 weeks to the CD-1 mouse at up to 7000 ppm was not carcinogenic and did not cause toxicity. The NOAEL was therefore set at 7000 mg/kg food (911.8 mg/kg bw/day).

Reproductive toxicity

One two-generation reproduction toxicity study in rat and two developmental toxicity studies in rat and rabbit were available. These studies are addressed in section 2.6.6 (for more detailed data please refer to RAR Volume 3, section B.6.6). For possible classification for STOT RE, only the parental or maternal toxicity in these studies might be of interest and concern.

In the rat, treatment-related findings were confined to high doses. This is shown by LOAELs for parental toxicity in the two-generation study that was 662.9 mg/kg bw/day based on reduced bodyweight gain in the F0 and F1 generation males and in the F0 females as well as reduced food consumption in the F1 generation males. In the developmental study conducted with Sprague-Dawley rats, no indication of maternal adverse toxicity was detected up to the international regulatory limit dose of 1000 mg/kg bw/day. Therefore, the maternal NOAELs were set at 1000 mg/kg bw/d, the highest dose tested.

Regarding rabbit developmental study, maternal LOAEL of 360 mg/kg bw/day was established. It was based on increased mortalities and retarded body weight gain to Day 15. There were no treatment-related clinical signs. At 360 mg/kg bw/day, one animal was found dead on day 13 (6 days after dosing) following a suspected convulsion and a second was killed on day 24 due to marked and continuing weight loss and was found to have haemorrhagic depressions in the stomach. Furthermore, the latter was aborted prior to sacrifice. It is noteworthy that there were 4/6 and 1/6 (unverified) mortalities in a preliminary study at 800 mg/kg bw/day and at 400 mg/kg bw/day, respectively. The mortalities in the preliminary studies were attributed to substance irritation of the stomach mucosa too as the animals had haemorrhagic depressions in the stomach.

Neurotoxicity

Rat subchronic 13 week neurotoxicity study was conducted and did not reveal any neuropathological or other adverse treatment-related findings up to 948 mg/kg bw/day. The study is addressed in section 2.6.7 (for more detailed data please refer to RAR Volume 3, section B.6.7.1.2.).

Immunotoxicity

The study conducted with trinexapac-ethyl in female mice did not reveal any signs of immunotoxicity when administered via the diet over a period of 28 days. The study is addressed in section 2.6.8.2 (for more detailed data please refer to RAR Volume 3, section B.6.8.2.1.).No clinical signs of systemic toxicity were observed in any dose groups (160.2, 613.7, 1630.5 mg/kg bw/day): there were no adverse effects on body weight, body weight changes or nutritional parameters. The NOAEL for immunotoxicity and systemic toxicity under the conditions of the present study in female mice was ≥ 1530.5 mg/kg bw/day, the highest concentration tested.

It should be noted that based on (Q)SAR analysis (using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited) submitted, trinexapac-ethyl did not trigger any Derek Nexus alert for endpoints relevant for repeated dose toxicity STOT RE (e.g. Bladder disorders, Bone marrow toxicity, Bradycardia, Cardiotoxicity, Cumulative effect on white cell count and immunology, Hepatotoxicity, Kidney disorders, Kidney function-related toxicity, Methaemoglobinaemia, Nephrotoxicity, Ocular toxicity, Pulmonary toxicity, Splenotoxicity, Thyroid toxicity and/or Urolithiasis). For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.

Table 49: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	**Target organ effect(s) (all significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed)**	**Effective dose (mg/kg/day)**	**Length of exposure**	**Guidance value/ Extrapolated guidance value when extrapolated to the exposure duration other than 90 days**	**Classification supported by the study (Cat 1, cat 2, NC)**
Oral study in rats (28 days)					
Anonymous, 1988 B.6.3.1.1	Liver and kidney for male ↑ water consumption (M, F); ↑absolute and relative liver (M, F) & kidney (M) weight; liver and kidney histopathology (M): hepatocellular hypertrophy in centrilobular regions of the liver and PAS-positive droplets in the epithelia of the collecting ducts of the kidneys.	1000/2000 mg/kg bw /d (M) **Not suitable to establish a proper LOAEL for females*	28 days	Cat 1: ≤30 mg/kg bw/day Cat 2:>30; ≤300 mg/kg bw/day	NC
Dermal study in rabbits (22 days)					
Anonymous, 1989 B..6.3.3.1	No systemic effects	>1000 mg/kg bw/d	22 days	Cat 1: ≤81 mg/kg bw/day Cat 2:>81; ≤810 mg/kg bw/day	NC
Oral study in dogs (7 weeks)					
Anonymous, (1989) B.6.3.2.4	General toxicity: ↓ body weight (M & F), ↓ percent body weight gain (M & F), ↓food consumption (M & F), non-specific secondary response: ↓ absolute and relative thymus weight & thymus atrophy (M & F) ↓bw (M & F): >10% at days 35-49) ↓FC (M: 30 - 79% days at 7, 14 and 28; F: >56% at days 35 - 49)	861 mg/kg bw /d		Cat 1: ≤18 mg/kg bw/day Cat 2:>18; ≤180 mg/kg bw/day	NC
Oral study in dogs (1 year)					

Anonymous, 1992 B.6.3.2.3 four supplementary studies: B.6.3.2.3.1; B.6.3.2.3.2; B.6.3.2.3.3; B.6.3.2.3.4	Clinical signs (faeces mucoid/bloody, M & F), ↓terminal bw (M: 11.5%), haematological changes (↓RBC, ↓HCT, ↓HGB) (F), changes in oestrus cyclicity, decreased absolute uterus weight, brain histopathology (cerebral vacuolation) (M & F)	357.1 mg/kg bw /d	1 year	Cat 1: ≤2.5 mg/kg bw/day Cat 2:>2.5; ≤25 mg/kg bw/day	NC
Combined chronic toxicity / carcinogenicity study in rats (52/104-week oral, dietary)					
Anonymous, 1992 B.6.5.1	Interim renal histopathological effects (hyaline droplets) (M) Bile duct hyperplasia in the liver (M), galactoceles in mammary skin (F)	392.7 mg/kg bw /d (M) 392.7 mg/kg bw /d (M & F)	Interim sacrifice (52 weeks) Chronic / Carcinogenicity (104 weeks)	Cat 1: ≤2.5 mg/kg bw/day Cat 2:>2.5; ≤25 mg/kg bw/day Cat 1: ≤1.2 mg/kg bw/day Cat 2:>1.2; ≤12 mg/kg bw/day	NC
Carcinogenicity study in mice (78-week oral)					
Anonymous, 1991 B.6.5.2	There were no adverse effects	> 911.8 mg/kg bw /d (M & F) (highest dose tested)	78 weeks	Cat 1: ≤1.6 mg/kg bw/day Cat 2:>1.6; ≤16 mg/kg bw/day	NC
Two-generation reproduction toxicity study in rats					
Anonymous, 1991 B.6.6.1.1	**Parental:** ↓bw gain premating (F0 males Day 0-91: 9.6%; F1 males Day 0-84: 10.5%; F0 female Day 0-91: 14.8%); ↓FC premating (F1 males: average 5.9%)	662.9 mg/kg/d	Approximate number of dose weeks: F0 – 22-25; F1 – 20-23	Approximately Cat 1: ≤6.0 mg/kg bw/day Cat 2:>6.0; ≤60 mg/kg bw/day	NC
Developmental toxicity (teratogenicity) study in rats					
Anonymous, 1988 B.6.6.2.1	**Maternal:** Did not cause adverse effects at highest dose tested.	> 1000 mg/kg (highest dose tested)	Exposure days 6-15 of gestation, gavage	Approximately Cat 1: ≤90 mg/kg bw/day Cat 2:>90; ≤900 mg/kg bw/day	NC
Developmental toxicity (teratogenicity) study in rabbits					

Anonymous, 1990 B.6.6.2.2	**Maternal:** ↑mortality (2/17): 1 animal on day 13 (6 days after dosing), second was killed on day 24 due to marked and continuing weight loss and was found to have haemorrhagic depressions in the stomach; retarded body weight gain to Day 15	360 mg/kg bw/d	Exposure days 7-19 of pregnancy, gavage	Approximately Cat 1: ≤ 69 mg/kg bw/day Cat 2:>69; ≤690 mg/kg bw/day	NC
Subchronic (13 week) dietary neurotoxicity study in rats					
Anonymous, 2012a B.6.7.1.2	No signs of neurotoxicity observed at highest dose tested. Did not cause systemic adverse effects at highest dose tested.	> 948 mg/kg bw/d (highest dose tested)	13 weeks	Cat 1: ≤10 mg/kg bw/day Cat 2:>10; ≤100 mg/kg bw/day	NC
28-Day immunotoxicity feeding study in mice					
Anonymous, 2011 B.6.8.2.1	No signs of immunotoxicity (the humoral and innate immune response) observed at highest dose tested. Did not cause systemic adverse effects at highest dose tested.	> 1530.5 mg/kg bw/d (highest dose tested)	28-days oral, dietary	Cat 1: ≤30 mg/kg bw/day Cat 2:>30; ≤300 mg/kg bw/day	NC

2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure)

Substances are classified in STOT RE Category 1 based on evidence of significant toxicity in humans or where there is evidence from studies in experimental animals that they can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. For classification in Category 1, either reliable good quality human data (evidence from human cases or epidemiological studies) or animal data (observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were observed at generally low exposure concentrations) is required. Annex I, Section 3.9.2.9.6 of the CLP Regulation provides a 'guidance value' of ≤10 mg/kg bw/day from a 90-day rat study to assist in Category 1 classification. For a 28 day study the guidance value of ≤30 mg/kg bw/day to assist in Category 1 classification.

Substances are classified in STOT RE Category 2 based on evidence from studies in experimental animals that they can be presumed to have the potential to be harmful to human health following repeated exposure. For classification in Category 2, animal data (observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were observed at generally moderate exposure concentrations) is required. Annex I, Section 3.9.2.9.7 of the CLP Regulation provides a 'guidance value' of 10-100 mg/kg bw/day from a 90-day rat study to assist in Category 2 classification. For a 28 day study the guidance value of ≤300 mg/kg bw/day to assist in Category 2 classification.

In the rat, the kidney and partly liver were the main target organs of toxicity. The kidney effects in males (increased kidney weight, PAS-positive droplets in the epithelia of the collecting ducts of the kidneys in males and scattered foci of tubular basophilia as well as cytoplasmic accumulations of hyaline droplets in cortical tubular epithelium) were considered relevant to humans. The histopathological kidney effects were noted at the high dose of 1000/2000 mg/kg bw/day for 28 days, 346 mg/kg bw/day for 90 days and at 392.7 mg/kg bw/day for 52-weeks. The liver effects in males (increased liver weight, hepatocellular hypertrophy in centrilobular regions and bile duct hyperplasia) were noted at the high dose of 1000/2000 mg/kg bw/day for 28 days and at 392.7 mg/kg bw/day for 2 years, respectively, but these liver effects were not confirmed in the 90-day study at similar dose levels. Therefore, in the rat, the only significant toxic effects of relevance to humans were seen in the kidney and partly in liver; however, these occurred at dose levels well in excess of the specified guidance values for classification with STOT-RE Category 2.

In the mouse, no significant toxic effects occurred at any dose: no treatment-related effects on any organ were seen in the carcinogenicity study up to dietary concentrations 911.8 mg/kg bw /day well in excess of the specified guidance values for classification with STOT-RE Category 2.

In the dog, there were no treatment-related effects up to the specified guidance values for rats. Decreased body weight, body weight gain and food consumption in both sexes, and emaciation in several males were seen from doses 890 mg/kg bw /day in the 90-day study. Clinical signs, microscopic evidence of brain histopathology (cerebral vacuolation) in both sexes, decreased terminal body weight in males, haematological findings (decreased RBC, haematocrit, haemoglobin) in females, changes in oestrus cyclicity and decreased absolute uterus weight were seen from doses 357.1 mg/kg bw /day in the 1-year study.

Low toxicity of trinexapac-ethyl upon repeated administration was confirmed in **carcinogenicity, reproductive toxicity** (two-generation study and developmental toxicity study in rat)**, neurotoxicity** and **immunotoxicity studies.**

No indication of maternal adverse toxicity was detected up to the international regulatory limit dose of 1000 mg/kg bw/day in the developmental study conducted with rats. The LOAEL concerning systemic toxicity for parental animals in the 2-generation rat study was 662.9 mg/kg bw/d based on reduced bodyweight gain in the F0 and F1 generation males and in the F0 females as well as reduced food consumption in the F1 generation males. In view of the fact that maternal LOAEL of 360 mg/kg bw/day was established in rabbit developmental study, the pregnant rabbit was more sensitive than rats to trinexapac-ethyl.

In **rabbit developmental study**, maternal LOAEL of 360 mg/kg bw/day was established and was based on increased mortalities and retarded body weight gain to Day 15. There were no treatment-related clinical signs. At 360 mg/kg bw/day, one animal was found dead on day 13 (6 days after dosing) following a suspected convulsion and a second was killed on day 24 due to marked and continuing weight loss and was found to have haemorrhagic depressions in the stomach. Furthermore, the latter was aborted prior to sacrifice. It is noteworthy that there were 4/6 and 1/6 mortalities in a preliminary study at 800 mg/kg bw/day and at 400 mg/kg bw/day, respectively. The mortalities in the preliminary studies were attributed to substance irritation of the stomach mucosa too as the animals had haemorrhagic depressions in the stomach.

It should be noted that there were no statistically significant and/or dose related differences in mean body weights and food consumption during treatment period (gestation days 7-19) and/or during gestation in all dose groups compared to controls. A characteristic of trinexapac-ethyl appears to be variability in the individual response regarding to body weight. Body weight gain of animals at 360 mg/kg/d dose was retarded relative to control, low and mid dose groups until Day 15: 13 females from 14 and 11 from 14 had reduced body weight gain on Day 9 and 11, respectively. However, these values did not attain statistical significance. It should be noted that two females in 360 mg/kg/d dose group showed depressed gains/loss throughout: one female did not recover and one female had regained the weight loss on Day 29.

For the evaluation of the rabbit developmental toxicity study, the findings at particular dose have been compared with guidance values corrected for the duration of the exposure (according to Haber's rule). It can be seen from the Table 49 that the only study in rabbits showed effects within the corrected guidance values for classification with STOT RE 2. However, it is important to take into account that guidance values are only for guidance purposes. However, there is a lack of information regarding whether the rabbits were able to eat their caecotrophes or not, and therefore it is not possible to have a clear picture of a possible recycling of active substance and consequently the actual dose absorbed from the GI tract, leading to uncertainties with using Haber's rule to correct the guidance value for a STOT RE classification in this study. According to CLP, Annex I, Section 3.9.2.9.8., "*The guidance values and ranges mentioned in paragraphs 3.9.2.9.6 and 3.9.2.9.7 are intended only for guidance purpose, i.e. to be used as part of weight of evidence approach, and to assist with decisions about classification. They are not intended as strict demarcation values."* Furthermore, an in-depth analysis of all the data from the short-term, all others reproductive toxicity, neurotoxicity and immunotoxicity studies doesn't show such effects as mortalities. All the data from the short-term, carcinogenicity, all others reproductive toxicity, neurotoxicity and immunotoxicity studies shows affects at high dose levels exceeding the non-extrapolated / extrapolated guidance values relevant for a classification with STOT RE.

Additionally, according to CLP, Annex I, Section 3.9.2.7.3, morbidity or death resulting from repeated or long-term exposure can be taken into account for classification as STOT RE. However, CLP further states that "*Morbidity or death resulting from repeated or long-term exposure, even to relative low doses/concentrations, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites."* Following exposure to trinexapac-ethyl, mortality in rabbits is considered to be related to substance irritation of the stomach mucosa as those animals had haemorrhagic depressions in the stomach. In addition, bioaccumulation and overwhelming of detoxification mechanisms by repeated exposure as a mechanism of toxicity is not likely for trinexapac-ethyl.

Hence, studies of repeated dose toxicity and carcinogenicity, neurotoxicity, reproductive toxicity as well as immunotoxicity studies with trinexapac-ethyl did not identify effects which constitute 'significant or severe toxicity' and were not seen at dose levels relevant to STOT RE classification.

2.6.3.1.3 Conclusion on classification and labelling for STOT RE (specific target organ toxicity-repeated exposure)

In the absence of any evidence of 'significant or severe toxicity' at low or generally moderate dose levels from repeated dose toxicity studies, trinexapac-ethyl does not require classification as STOT RE.

2.6.4 Summary of genotoxicity / germ cell mutagenicity

Table 50: Summary table of genotoxicity/germ cell mutagenicity tests *in vitro*

Method, guideline, deviations[1] if any	**Test substance (Batch No; purity)**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
Bacterial Reverse Mutation Test (Ames test) OECD 471 (1983) GLP The study is considered acceptable.	Trinexapac-ethyl, P.705002, 96.6%	Organism/ Strain(s): *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 Concentrations tested (range): 20 - 5000 µg/plate (-/+ S9).	Negative	Deparade, 1988
Bacterial Reverse Mutation Test (Ames test) OECD 471 (1997 GLP The study is considered acceptable.	Trinexapac-ethyl, P.306042, 96.8%	Organism/ Strain(s): *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535, TA 1537, *Escherichia coli* WP2 uvrA Concentrations tested (range): 20 - 5000 µg/plate (-/+ S9).	Negative	*Deparade, 2001a*
Bacterial Reverse Mutation Test (Ames test) OECD 471 (1997) GLP The study is considered acceptable.	Trinexapac-ethyl, SMO7J020, 95.6%	Organism/ Strain(s): *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2 uvr A pKM 101 WP2 pKM 101 Concentrations tested (range): 3-5000 µg/plate (-/+ S9).	Negative	*Sokolowski, 2010*
Bacterial Reverse Mutation Test (Ames test) OECD 471 (1997) GLP The study is considered acceptable.	Trinexapac-ethyl, SMO5D180 (fortified) 93.3%	Organism/ Strain(s): *Salmonella typhimurium* TA1535, TA1537, TA98, TA100 *Escherichia coli* WP2 *uvr*A (pKM101) Concentrations tested (range): 5-5000 µg/plate (-/+ S9) – Plate incorporation assay; 15-5000 µg/plate (-/+ S9) – Preincubation test	Negative	*Woods, 2017*
Bacterial Reverse Mutation Test (Ames test) OECD 471 (1997) GLP The study is considered acceptable.	Trinexapac-ethyl, 201111003, 98%	Organism/ Strain(s): *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537 *Escherichia coli* WP2 uvrA	Negative	*Schreib, 2015*

Method, guideline, deviations[1] if any	**Test substance (Batch No; purity)**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
		Concentrations tested (range): 0.013-5.0 µL/plate (-/+ S9)		
Bacterial Reverse Mutation Test (Ames test) OECD 471 (1997) GLP The study is considered acceptable.	Trinexapac-ethyl, 200711001, 98.1%	Organism/ Strain(s): *Salmonella typhimurium* *TA 98,* *TA 100,* *TA102* *TA 1535,* *TA 1537* Concentrations tested (range): 156.25-5000 µg/plate (-/+ S9)	Negative	*Williams, 2009*
Bacterial Reverse Mutation Test (Ames test) OECD 471 (1997) GLP The study is considered acceptable.	Trinexapac-ethyl, CSO-1282-TE-29 98.8 %	Organism/ Strain(s): *Salmonella typhimurium:* *TA100,* *TA1535, TA1537,* *TA98* *Escherichia coli* WP2 uvrA Concentrations tested (range): 31.6-5000 µg/plate (-/+ S9)	Negative	*Donath, 2011*
Bacterial Reverse Mutation Test (Ames test) OECD 471 (1997) GLP The study is considered acceptable.	Trinexapac-ethyl, 201111005, 98.1%	Organism/ Strain(s): *Salmonella typhimurium:* *TA100,* *TA102,* *TA1535, TA1537,* *TA98* Concentrations tested (range): 31.6-5000 µg/plate (-/+ S9)	Negative	*Spruth, 2015*
Bacterial Reverse Mutation Test (Ames test) OECD 471 (1997) GLP The study is considered acceptable.	Trinexapac-ethyl, 201309001, 98.03%	Organism/ Strain(s): *Salmonella typhimurium:* *TA100,* *TA1535, TA1537,* *TA98* *Escherichia coli* *WP2 uvrA* Concentrations tested (range): 31.6-5000 µg/plate (-/+ S9)	Negative	*Schreib, 2014*
Mammalian Cell Gene Mutation Test (HPRT) OECD 476 (1984) GLP The study is considered acceptable.	Trinexapac-ethyl, P.705002, 96.6%	Organism/ Strain(s): Chinese hamster V79 cells Concentrations tested (range): 70 - 1400 µg/ml (-/+ S9).	Negative Cytotoxicity 1500 µg/ml (+S9); >1500 µg/ml (-S9)	*Dollenmeier, 1988*
In vitro Mammalian Cell Gene Mutation Test (TK) OECD 476 (1984)	Trinexapac-ethyl, P.001010, 94.5%	Organism/ Strain(s): Mouse lymphoma cells L5178Y	Negative	*Geleick, 1993*

Method, guideline, deviations[1] if any	Test substance (Batch No; purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
GLP The study is considered acceptable.		Concentrations tested (range): 7.54 - 1930 µg/ml (-/+S9).		
In vitro Mammalian Cell Gene Mutation Test (HPRT) OECD 476 (2016) GLP The study is considered acceptable.	Trinexapac-ethyl, SMO5D180 (fortified), 93.3%	Organism/ Strain(s): Chinese hamster ovary (CHO-K1) cells Concentrations tested (range): 43.75 - 1400 µg/mL (-/+S9)	**Without activation:** **Positive** 175.0 and 1400.0 - Main experiment **Negative** Confirmatory experiment **With activation:** Negative **Remark:** ≥1600 µg/mL fluctuations in pH of more than 1.0 unit Results **equivocal**	*Gilby, 2017*
In vitro Mammalian Cell Gene Mutation TK Test OECD 476 (1997) GLP The study is considered acceptable.	Trinexapac-ethyl, 200711001, 98.1 %	Organism/ Strain(s): Mouse lymphoma cells L5178Y Concentrations tested (range): 250 to 2523 µg/ml (-/+S9)	**Without activation:** Negative **With activation:** **Positive** (at 2523 µg/ml) **Remark:** Cytotoxicity 2523 µg/ml (+S9) Results **equivocal**	*Stone,2009*
Mammalian Chromosome Aberrations Test OECD 473 (1997) GLP The study is considered acceptable.	Trinexapac-ethyl, P.306042, 96.8%	Organism/ Strain(s): Chinese hamster ovary (CHO K5) cells Concentrations tested (range): 312.5 - 1250 µg/ml (-/+ S9)	Negative	*Ogorek, 2001*
Mammalian Chromosome Aberrations Test OECD 473 (2014) GLP	Trinexapac-ethyl, SMO5D1422, 95.7%	Organism/ Strain(s): Human lymphocytes Concentrations tested (range): 491.9 - 1506.3 µg/ml (-/+S9) 4h exposure;	Negative	*Sokolowski, 2015*

Method, guideline, deviations[1] if any	Test substance (Batch No; purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
The study is considered acceptable.		281.1 - 1506.3 µg/ml (-S9) 22 h exposure		
Mammalian Chromosome Aberrations Test OECD 473 (1997) GLP The study is considered acceptable.	Trinexapac-ethyl, 200711001, 98.1 %	Organism/ Strain(s): Human lymphocytes Concentrations tested (range): Experiment **1** 800 - 2523 µg/ml (-S9) 3+17h exposure 1200 - 2523 µg/ml (+S9) 3+17h exposure Experiment **2** 200 - 600 µg/ml (-S9) 20+0h exposure 1700 - 2523 µg/ml (+S9) 3+17h exposure Experiment **3** 50 - 650 µg/ml (-S9) 20+0h exposure 1700 - 2523 µg/ml (+S9) 3+17h exposure	**Without activation:** Negative **With activation: Positive** (at 2523 µg/ml) in Experiment 1 **Remark:** Results **equivocal**	*Lloyd, 2010*
DNA Damage and Repair Test OECD 482 (1986) GLP The study is considered acceptable.	Trinexapac-ethyl, P.705002, 96.6%	Organism/ Strain(s): Primary rat hepatocytes Concentrations tested (range): 0.8 - 400 µg/ml (- S9) 4 - 500 µg/ml (- S9)	Negative Cytotoxicity 328 µg/ml	*Hertner, 1988*
DNA Damage and Repair Test OECD 482 (1986) GLP The study is considered supplementary study due to some deviations.	Trinexapac-ethyl, P.705002, 96.6%	Organism/ Strain(s): Human fibroblasts Concentrations tested (range): 37.04 - 4000 µg/ml (- S9)	Negative Cytotoxicity 5250 µg/ml	*Meyer, 1988*

Table 51: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations[1] if any	Test substance (Batch No; purity)	Relevant information about the study (as applicable)	Observations/Results	Reference
Micronucleus Test OECD 474 (1983) GLP The study is considered to be supplementary due to the limitations in experimental design and reporting.	Trinexapac-ethyl, P.705002, 96.6%	Species: Mouse, Tif: MAGF, SPF Frequency of application: single dose (orally) Concentrations tested (range): Study 1: 3000 mg/kg bw, sacrificed 16, 24 and 48 h Study 2: 750, 1500, 3000 mg/kg bw sacrificed 48 h	Negative Toxicity 3000 mg/kg	Anonymous, 1989 B.6.4.2.1 study 1

Method, guideline, deviations[1] if any	Test substance (Batch No; purity)	Relevant information about the study (as applicable)	Observation s/Results	Reference
Micronucleus Test OECD 474 (1983) GLP The study is considered acceptable.	Trinexapac-ethyl, P.001010, 94.5%	Species: Mouse, Tif: MAGF, SPF Frequency of application: single dose (orally) Concentrations tested (range): 1000, 2000, 4000 mg/kg bw sacrificed 16, 24 and 48 h	Negative Toxicity 4000 mg/kg	Anonymous, 1992 B.6.4.2.1 study 2
Micronucleus Test OECD 474 (1997) GLP The study is considered acceptable.	Trinexapac-ethyl, CSO-1282-TE-29 (200911007)/ 98.8%	Species: Rat (male), Sprague Dawley Frequency of application: Single dose (two administrations/24 hours), (orally) Concentrations tested (range): 350, 700, or 1400 mg/kg bw/day sampled for bone marrow analyses 24 hours after the final administration	Negative 1400 mg/kg bw/day (MTD)	Anonymous, 2010 B.6.4.2.2

Table 52: Summary table of human data relevant for genotoxicity / germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

Trinexapac-ethyl (CGA 163935) was tested in many genotoxicity and mutagenicity tests *in vitro* and *in vivo*.

CGA 163935 did not induce point mutations in bacteria *in vitro*. In mammalian cells *in vitro*, the results of 2 gene mutation tests, a chromosome aberrations assay and 2 unscheduled DNA synthesis (UDS) assays were negative. One chromosome aberrations assay (*Strasser F., 1989*) was rejected on the grounds of guideline deviations. Two *in vivo* mouse micronucleus tests likewise had negative results. Re-evaluation of all studies has been performed by the RMS: conclusions have not been changed, however, one unscheduled DNA synthesis (UDS) assay (*Meyer A., 1988*) and one *in vivo* study (Anonymous, *1989*) (B.6.4.2.1 study 1)) were considered to be supplementary due to deviation with regard to experimental design.

Since Annex I of Council Directive 91/414/EEC inclusion two new genotoxicity studies have been conducted on trinexapac-ethyl technical (Syngenta) in order to establish the equivalence of the impurity profiles: Bacterial reverse mutation assay (Ames test) and *in vitro* chromosome aberration study. Trinexapac-ethyl technical was tested in *Salmonella typhimurium* and in *Escherichia coli* strains. Results of the study indicate that Trinexapac-ethyl technical was not mutagenic in this bacterial mutation test either in the absence or in the presence of exogenous metabolic activation. Trinexapac-ethyl technical did not induce chromosome aberrations in human lymphocytes *in vitro*. Hence, there was no evidence of point mutations in the Ames study, and no evidence of clastogenicity in the *in vitro* chromosome aberration study. The new genotoxicity studies are in agreement with previously conclusion that trinexapac-ethyl technical (Syngenta) is unlikely to be genotoxic.

Trinexapac-ethyl (CGA 163935) did not induce point mutations in bacteria *in vitro*. In mammalian cells *in vitro*, the results of 2 gene mutation tests, a chromosome aberrations assay and 2 unscheduled DNA synthesis (UDS) assays were negative. In addition, it should be noted that one chromosome aberrations study was not acceptable (Strasser, 1989) due to serious deviations from OECD 473 (1983) (for more detailed data please refer to RAR Volume 3, section B.6.4.1.2). Two deviations from OECD guideline 482 were noted in unscheduled DNA synthesis (UDS) assay in primary mammalian cells assay (Meyer, 1988). The cells were not exposed to test substance with metabolic activation. In addition, the selection of 4000 µg/ml as the highest concentration in the DNA repair test is justified only as being the best suited, although cytotoxicity was only evident in the cytotoxicity test at one concentration (5250 µg/ml). However, taking into account the high concentrations used in the main test, this deviation is not considered a serious one.

Two *in vivo* mouse micronucleus tests are available and likewise had negative results (Anonymous, 1992 B.6.4.2.1 study 2, and Anonymous, 1989 B.6.4.2.1 study 1). The latter study report (Anonymous, 1989) was checked for compliance with OECD 474 (adopted 29 July 2016 and/or 21 July 1997) and it was concluded that the study does not appear to comply with the updated OECD guideline. Therefore, the study was considered to be supplementary due to the limitations in experimental design and reporting (for more detailed data please refer to RAR Volume 3, section B.6.4.2.1). Although the second study (Anonymous, 1992) is considered to be acceptable, it does not fulfil current data requirements. This study design is limited since it could not be shown that target tissue is reached. Though the dose levels used in this study were very high (limit 2000 mg/kg bw/day dose and MTD exceeded dose), no cytotoxicity was seen in bone marrow. On the other hand, it could be assumed that the substance administered reached the bone marrow. The assumption could be partly based on the findings from the ADME studies in other species (rats) where distribution of trinexapac-ethyl in bones, blood and plasma, after single oral low (1 mg/kg bw) and high (~200 mg/kg bw) dose administration was detected (Anonymous, 1995 (B.6.1.1 Study 2)). In addition, bone showed the longest slow phase half-life ($T_{1/2}$), after low and high dose administration: 3.2 h and 12 h, respectively. No increases in the number of micronuclei in polychromatic erythrocytes were observed in this study. Taken all together, performing a new study is not considered necessary.

In addition, in response to comment at renewal, the applicant (Syngenta) has submitted Ames and HPRT assays (*Woods I., 2017* and *Gilby B., 2017*) with spiked batch material to support the technical specification. Trinexapac-ethyl tech. fortified did not induce point mutations in bacteria *in vitro*. However, the result of gene mutation assay was equivocal and whether the assay support the technical specification is considered in the confidential Volume 4 Syngenta, C.1.4.2.

In response to comment at renewal, the applicant (Adama) has submitted an Ames test on the active substance (*Schreib G., 2015*) in order to establish the equivalence of the impurity profile. Results of the study indicate that Trinexapac-ethyl technical was not mutagenic in this bacterial mutation test with and without metabolic activation.

In response to comment at renewal, the RMS LT updated RAR with five genotoxicity studies provided by the applicant Cheminova A/S (the company of a Task Force). There was no indication of induction of gene mutation either in the presence or absence of metabolic activation in the two bacterial reverse mutation assays (*Donath C., 2011; Williams L., 2009*). The gene mutation test in mouse lymphoma cells was positive in the presence of metabolic activation but only at concentrations where marked toxicity was observed (*Stone V., 2009*). Trinexapac-ethyl tech. seems to have particular genotoxic effect in the mammalian chromosome aberrations assay *in vitro* (*Lloyd M., 2010*) under metabolic-activation conditions. However, a rat micronucleus test *in vivo* (Anonymous, *2010* (B.6.4.2.2)) gave the negative result: the test material did not induce micronuclei in bone marrow of rats and sufficient evidence of bone marrow exposure was demonstrated from toxicokinetic studies.

In response to comment at renewal, the applicant (Helm) has submitted robust summaries of two Ames tests on the active substance (*Spruth B., 2015; Schreib G., 2014*). Trinexapac-ethyl tech. did not induce gene mutations with and without metabolic activation.

It should be noted that based on (Q)SAR analysis (using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited) submitted, trinexapac-ethyl did not trigger in any Derek Nexus structural alert for genotoxicity (e.g. Mutagenicity *in vivo/in vitro*, Chromosome damage *in vitro/in vivo*, Non-specific genotoxicity *in vitro/in vivo*). For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.

In conclusion, trinexapac-ethyl did not have genotoxic effects on bacteria *in vitro*, or on mammalian cells *in vivo*.

All nine AMES tests were negative. Although studies of Stone V. (2009) and Gilby B. (2017) are considered equivocal, further *in vivo* studies are not justified. Overall, considering available data on AMES test and mammalian gene mutation, the compound is unlikely to be of gene mutation concern. Taking into account the negative results of the *in vivo* MN test there is no concern for chromosome aberration *in vivo*. Based on a weight of evidence of all data available trinexapac-ethyl does not pose genotoxic concern *in vivo*.

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

Annex I Section 3.5.1.1 of the CLP regulation defines mutation as a permanent change in the amount or structure of the genetic material in a cell. The term ‘mutation‘ applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications. The term ‘mutagenic’ and ‘mutagen’ are used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms. This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from mutagenicity or genotoxicity tests *in vitro* and in mammalian somatic and germ cells *in vivo* are also considered in classifying substances within this hazard class.

Classification for mutagenicity in Category 1 is appropriate for substances known to induce heritable mutations (Category 1A) or for substances regarded as if they induce heritable mutations in the germ cells of humans (Category 1B).

Classification in Category 1A is based on positive evidence from human epidemiological studies.

Classification in Category 1B is based on positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with evidence that the substance has potential to cause mutations to germ cells; or positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny.

Classification for mutagenicity in Category 2 is appropriate for substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. Classification in Category 2 is based on positive evidence obtained from somatic cell mutagenicity tests in mammals and/or in some cases from somatic cell mutagenicity tests in mammals and supporting data from *in vitro* experiments.

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

Overall, considering available data on AMES test and mammalian gene mutation, the compound is unlikely to be of gene mutation concern. Based on negative micronucleus tests *in vivo* where sufficient evidence of bone marrow exposure was demonstrated from toxicokinetic studies, trinexapac-ethyl is unlikely to be genotoxic *in vivo*. The criteria for classification for mutagenicity were not met. On the basis of the available data, no hazard classification of trinexapac-ethyl for mutagenicity is warranted according to Regulation (EC) No 1272/2008.

2.6.5 Summary of long-term toxicity and carcinogenicity

Table 53: Summary table of animal studies on long-term toxicity and carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (Batch No; purity), dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Combined chronic toxicity /carcinogenicity study OECD 453 (1981) GLP Rat, Sprague-Dawley [Crl:VAF/Plus CD (SD) Br] Chronic (104 weeks): 20/sex/dose Carcinogenicity (104 weeks): 50/sex/dose Interim sacrifice (52-weeks): 10/sex/dose Interim recovery (52 + 4-weeks recovery): 10/sex/dose (control and 20000 ppm) The study is considered acceptable, despite some deviations.	Trinexapac-ethyl, FL 872026, 96.9% FL 881224, 96.9% FL 882373, 96.2% FL 892178, 96.2% FL 891417, 92.2% 0, 10, 100, 3000, 10000, 20000 ppm Equal to 0, 0.4, 3.9, 115.6, 392.7, 805.7 mg/kg bw/d for males and 0, 0.5, 4.9, 147.4, 494.0, 1054.0 mg/kg bw/d for females 52/104-week oral, dietary	Long-term NOAEL = 115.6 mg/kg bw /d (M & F) Long-term LOAEL= 392.7 mg/kg bw /d (M & F), based on interim renal histopathological effects (hyaline droplets) and bile duct hyperplasia in the liver (M), galactoceles in mammary skin (F) NOAEL for carcinogenicity ≥ 805.7 mg/kg bw /d (M & F) **Thyroid follicular adenocarcinoma** at 20000 ppm (♂4/80*; 5%); HCD (M): average 1.8 %, incidence range 0.0-5.0% **Squamous cell carcinoma** in the non-glandular stomach at 20000 ppm (♂2/80*; 2.5%); HCD (0%) **Urinary bladder papilloma** at 20000 ppm (♀2/80*; 2.5%); HCD (0%) ** Statistically significant difference from control group mean at the p-value 0.05 level* An increase incidence of thyroid follicular adenocarcinoma, squamous cell carcinoma in the non-glandular stomach (M) and papilloma of the urinary bladder (F) was considered as incidental	Anonymous, 1992 B.6.5.1
Carcinogenicity study OECD 451 (1981) GLP	Trinexapac-ethyl, FL 872026, 96.9% FL 881224, 96.9% FL 882373, 96.2%	Long-term NOAEL ≥ 911.8 mg/kg bw /d (M & F) (highest dose tested) There were no adverse effects	Anonymous, 1991 B.6.5.2

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (Batch No; purity), dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Mouse, Crl:CD-1(ICR)Br 70/sex/dose The study is considered acceptable.	0, 7, 70, 1000, 3500, 7000 ppm Equal to 0, 0.91, 9.01, 130.81, 450.72, 911.77 mg/kg bw/d for males and 0, 1.08, 10.66, 154.08, 538.73, 1073.42 mg/kg bw/d for females 78-week oral, dietary	NOAEL for carcinogenicity ≥ 911.8 mg/kg bw /d (M & F) (highest dose tested) There were no tumour incidence	

Table 54: Summary table of human data on long-term toxicity and carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 55: Summary table of other studies relevant for long-term toxicity and carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
(Q)SAR analysis using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited)	Trinexapac-ethyl (CGA 163935)	The results of (Q)SAR analysis) on parent substance were submitted (16-03-2017) on the request by the RMS. The software compares the structures with known toxicity endpoints. When a structural alert is identified the programme assigns a probability to the expression of toxicity by the compound.	Trinexapac-ethyl did not trigger Derek Nexus alert for 'Carcinogenicity' endpoint. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.	Anonymous, 2017*

** - Syngenta has requested data confidentiality for these data pursuant to art.63 of Reg. (EC) 1107/2009*

2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity

One 52/104-week combined chronic toxicity and carcinogenicity study in the rat and one 78-week study in the mouse have been previously submitted (for more detailed data please refer to RAR Volume 3, section B.6.5.1. and section B.6.5.2., respectively).

In the first **104-wk combined chronic toxicity /carcinogenicity study** (Anonymous, 1992 (B.6.5.1)) in rats exposed to up to 20000 ppm (805.7 mg/kg bw/day for males and 1054.0 mg/kg bw/day for females), mortality was >50% in all dose groups except the male high dose group. Statistically significant reductions (>10%) in mean body weight, percent body weight gain and food consumption occurred intermittently in males and females at 20000 ppm throughout the study but not at study termination. Hence, there were no adverse effects on mean body weight, percent body weight change and food consumption at concentrations ≤10000 ppm (392.7 mg/kg bw/day for males and 494.0 mg/kg bw/day for females).

The NOAEL for long-term effects was set at 3000 ppm (115.6 mg/kg bw/day for males and 147.4 mg/kg bw/day for females), based on an increase in the incidence of bile duct hyperplasia in the livers of males and galactoceles in mammary skin of females at the next higher dose level. In addition, following the initial 52 weeks of the study renal histopathological effects (hyaline droplets) were observed in 10000 ppm and 20000 ppm males.

An increased incidence of rare tumours was recorded following chronic exposure of Sprague-Dawley rats to trinexapac-ethyl. At 20000 ppm (805.7 mg/kg bw for males and 1054.0 mg/kg bw for females), males developed squamous cell carcinoma in the non-glandular stomach and thyroid follicular adenocarcinoma, whereas urinary bladder papilloma were increased in females.

The historical control data (HCD) for the tumour incidences were submitted by the notifier and almost covered a three-year period: the six studies were conducted between September 1984 and March 1987. HCD fell within a period of up to around 5 years of the present study as it was conducted between October 1988 and November 1990. In addition, the same strain of rats (Sprague-Dawley) from the same source (Charles River Laboratories Kingston, New York, USA) was used.

A statistically significant increase in the incidence of *urinary bladder papilloma* was found in two females at 20000 ppm (2/80; 2.5%). and in one 1-year female at 3000 ppm (not statistically significant). There was a statistically significant trend in the incidence (p=0.013; 0, 0, 0, 0, 1, 2). There was the same neoplastic finding in one 2-year male at 3000 ppm only. This spontaneous tumour was found in one “early deaths” female and in one 2-year female at 20000 ppm. This particular type of benign tumour (urinary bladder papillomas) is infrequently observed in rats and that was reflected by HCD from the conducting laboratory (0%). If the term papilloma is interchangeable in this case with polyp, transitional cell papillomas, and transitional cell polyp, the following historical data are available:

Historical control incidence of urinary bladder polyp:

Cumulative incidences / Total number of sites examined		Cumulative incidence (%)		Individual study Incidence range (%)	
M	F	M	F	M	F
0/389	1/389	0.0	0.3	0.0 – 0.0	0.0 – 1.4

Historical control incidence of transitional cell carcinoma in urinary bladder:

Cumulative incidences / Total number of sites examined		Cumulative incidence (%)		Individual study Incidence range (%)	
M	F	M	F	M	F
1/389	1/389	0.3	0.3	0.0 – 1.7	0.0 – 1.4

Urinary bladder polyps in females are recorded in these HCD with a spontaneous frequency of between 0-1.4%.

The incidence of pre-neoplastic lesions (e.g. a common pre-neoplastic finding such as epithelial hyperplasia in the urinary bladder) in the present study was observed in both sexes, however it was not considered to be compound-related: the more especially as it did not occur in the higher dose females. There were no transitional cell carcinomas observed in the female, however a transitional cell carcinoma was reported in control group male. The incidence was tabulated in the report as follows:

Summary of proliferative lesions

Males:

Group, Dose Level (ppm)	**1 0**	**2 10**	**3 100**	**4 3000**	**5 10000**	**6 20000**
Number examined	90	80	80	80	80	80
Epithelial hyperplasia	1	4	0	4	0	2
Papilloma	0	0	0	1	0	0
Transitional cell carcinoma	1	0	0	0	0	0
Total	2	4	0	5	0	2

Females:

Group, Dose Level (ppm)	**0**	**10**	**100**	**3000**	**10000**	**20000**
Number examined	89	80	80	80	80	80
Epithelial hyperplasia	2	2	0	1	1	0
Papilloma	0	0	0	0	1	2
Transitional cell carcinoma	0	0	0	0	0	0
Total	2	2	0	1	2	2

One of the possible non-genotoxic modes of action, by which urinary bladder tumours in rodents may be produced, is the presence of solid aggregates within the urinary tract. The relevance of this tumour to humans is probably low-moderate: anatomical differences between rodents and human bladder decrease the likelihood of prolonged residence of uroliths in human bladder, but there is still an epidemiological association between urinary tract stones and cancer (WHO, IARC Scientific Publications No. 147, Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis 1999) . It should be noted that one 20000 ppm female and one 3000 ppm male had macroscopic calculi (stones) observed in the urinary bladder at examination post mortem. These were considered to have led to the development of the papillomas. The calculi were removed before sectioning and would not have been present in the stained section, therefore were not described histologically. Hence, two of the tumours had distinct causes that were probably unrelated to administration of trinexapac-ethyl. Excluding these two tumours, the urinary bladder papilloma incidences are 0, 0, 0, 0, 1 (1.25%) and 1 (1.25%) in Groups of females 1 to 6, respectively. Excluding these two tumours, the overall tumour incidences are 1, 0, 0, 0, 1 and 1 in Groups 1 to 6, respectively.

Additionally, according to the notifier considering total proliferative changes in the bladder does not reveal any effects of treatment at any dose level. If all the proliferative lesions of the bladder are added together, the incidences were 4, 6, 0, 6, 2 and 4 or 4, 6, 0, 5, 2 and 3 without the animals with calculi, indicating that trinexapac-ethyl is unlikely to have carcinogenic potential.

The increased incidence of urinary bladder papilloma was considered as incidental based on an overall weight and strength of evidence approach.

A statistically significant increase in the incidence of *thyroid follicular adenocarcinoma* was observed in males at 20000 ppm (4/80; 5%). There was a statistically significant positive trend in the incidence (p = 0.042; 1, 0, 0, 1, 1, 4). This finding was observed in the control group (1/89) and in the other two lower dose groups (1/80) of males and in 1000 ppm females. An increased incidence of the thyroid follicular adenocarcinoma at the top dose level was just at the upper edge of HCD (2-year) range given and was above the average of HCD.

Historical control incidence of thyroid follicular adenocarcinoma (2-year):

Cumulative incidences / Total number of sites examined		Cumulative incidence (%)		Individual study Incidence range (%)	
M	F	M	F	M	F
7/389	7/388	**1.8**	1.8	**0.0 – 5.0**	0.0 – 4.3

On the other hand, this type of tumour was generally found at terminal sacrifice only (1, 0, 0, 0, 1, 4), i.e. increased in incidence with age. The incidence of thyroid follicular adenocarcinoma in 2-year males only (excluding interim necropsy) was slightly outside of historical range (4/70, 5.7%). A relationship of increased incidences of this type of tumour in male rats to a statistically significant increase in survival rate of males (32.5%) at the highest dose cannot be totally discounted.

The incidence of thyroid follicular neoplasm in male rats was tabulated in the report as follows:

Dose Level (ppm)	**0**	**10**	**100**	**3000**	**10000**	**20000**	**Historical Control**
Number of Animals	89	79	80	80	80	80	
Follicular adenomas (number/%)	4 (4.5%)	2 (2.5%)	3 (3.75%)	5 (6.25%)	3 (3.75%)	3 (3.75%)	0-8.6%
Follicular adenocarcinomas	1 (1.1%)	0	0	1 (1.25%)	1 (1.25%)	4 (5%)	0-5%
Combined	5 (5.6%)	2 (2.5%)	3 (3.75%)	6 (7.5%)	4 (5%)	7 (8.75%)	2-13%

The incidence of adenomas or the combined incidence of adenoma and adenocarcinomas showed no dose-related increase. Other lesions indicating an effect on the thyroid gland such as hypertrophy have not been reported in this study or in other toxicity studies with the test substance, and pre-neoplastic lesions (such as follicular hyperplasia of the thyroid) in the present study were seen in similar incidences in all groups. No neoplastic effect was seen in the thyroids of females and others species.

The thyroid follicular cell is one of the more common target sites for tumorigenesis in long-term toxicological studies in rats. Both genotoxic and non-genotoxic agents have been shown to induce thyroid follicular-cel tumours. There are several species differences in thyroid physiology. The lack of thyroid-binding globulin (TBG) in the adult rats is an important difference. Major differences are also present in the half-life thyrosine and in the serum level of thyroid-stimulating hormone (TSH) which is more than 25 times higher in the rodent than in human. The weight of the evidence suggests that rodents are more sensitive than human subjects to thyroid tumour induction due to hormonal imbalance that cause elevated TSH level. Agents that induce thyroid tumours in rodents through interference with thyroids hormone homeostasis can, with few exceptions, also interfere with thyroid hormone homeostasis in humans if given at a sufficient dose for a sufficient time. These agents can be assumed not be carcinogenic in humans at exposure levels which do not lead to alterations in thyroid hormone homeostasis. Hence, due to several species differences in thyroid physiology the relevance of this tumour to humans in the case of non-genotoxic mode of action is low (WHO, IARC Scientific Publications No. 147, Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis 1999).

The increased incidence of thyroid follicular adenocarcinoma was considered as incidental.

A statistically significant increase in the incidence of *squamous cell carcinoma in the non-glandular stomach* was reported in males at 20000 ppm (2/80; 2.5%) only. Though the incidence was above HCD from the conducting laboratory (0%), the number of affected rats was very low, limited by one sex and only the highest dose level. The incidence for this spontaneous tumour was exclusive observed in "early deaths" males and therefore cannot be linked to an older age and an increase in survival rate of males at the highest dose. According to the notifier other published data indicate that squamous cell carcinoma of the stomach is a rare spontaneous tumour (0 - 1.2% incidence).

The incidences in basal epithelial cell hyperplasia of the non-glandular stomach (pre-neoplastic lesion) in both sexes were not considered to be compound related. Pre-neoplastic findings such as acanthosis of the non-glandular stomach were found in females. It should be noted that non-glandular forestomach does not have an equivalent in human and the relevance of this tumour to humans is probably low. The increased incidence of squamous cell carcinoma in the non-glandular stomach was considered as incidental based on an overall weight and strength of evidence approach.

In conclusion, tumours occurred at the highest dose, at which the maximal tolerated dose (MTD) was not clearly reached. The tumour incidences were either just at the upper edge of HCD range given or above. The incidence of tumours was low and/or very low, tumours occurred at a number of apparently unrelated sites, in one species only and at high doses, not otherwise considered excessively toxic. A statistically significant increase in the incidence of tumour was reported in one sex only. Trinexapac-ethyl is unlikely genotoxic *in vivo*. There is no evidence of carcinogenicity in the second species tested. All three types of tumours have low or probably low-moderate relevance to humans. There was no evidence of pre-neoplastic changes in any of the tumour-bearing organs. These increased incidences of tumours were considered not to be related to treatment with trinexapac-ethyl and therefore, trinexapac-ethyl was not carcinogenic under the conditions of this study. Hence, the NOAEL for carcinogenicity was 20000 ppm (805.7 mg/kg bw/day), the highest dose tested. On balance, no classification is proposed.

There were no clinical signs of toxicity and any treatment-related effects on survival (≥50% in all dose groups), haematology, ophthalmology, organ weights or macroscopic findings in the second **78 weeks carcinogenicity study in mice** (Anonymous, 1991 (B.6.5.2)). Although it complied with the guideline requirement current at the time it was performed, the study failed to meet the requirement for the updated OECD 451 (adopted 7 September 2009): under the conditions of this study, the maximal tolerated dose (MTD) seems not to be reached. The observed effects on body weight, percent body weight gain and food consumption were not considered to be adverse. The increased incidences of several minor modifications in the normal lesions of ageing mice (amyloidosis and abscesses) at high doses were not considered to be of either adverse character or treatment-related, respectively.

No compound-related increases in the incidence of any tumours were observed in this study and trinexapac-ethyl was not considered to be carcinogenic in this strain of mice under the conditions of the study.

Dietary administration of trinexapac-ethyl for 78 weeks to the CD-1 mouse at up to 7000 ppm was not carcinogenic and did not cause toxicity. The NOAEL was therefore set at 7000 mg/kg food (911.8 mg/kg bw/day). No classification is proposed.

It should be noted that based on (Q)SAR analysis (using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited) submitted, trinexapac-ethyl did not trigger in any Derek Nexus structural alert for carcinogenicity. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

Annex I Section 3.6.1.1 of the CLP Regulation defines a carcinogen as a substance which induces cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans. Carcinogenic substances are allocated to Category 1 (known or presumed human carcinogens) or Category 2 (suspected human carcinogens).

A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. Substances known to have carcinogenic potential for humans (based largely on human evidence) are classified in Category 1A. Substances presumed to have carcinogenic potential for humans (based largely on animal evidence) are classified in Category 1B. In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

Studies performed with trinexapac-ethyl in the rat and mouse do not provide sufficient evidence of carcinogenicity based on an overall weight and strength of evidence approach and in consideration of the important factors in Annex I section 3.6.2.2.6 of the CLP Regulation.

Table 56: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Rat, Sprague-Dawley	Urinary bladder papilloma at 20000 ppm	Yes**:	Yes**:	No one "early deaths",	females	MTD was not reached at the highest dose	oral, dietary	The possible presence of solid

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
[Crl:VAF/ Plus CD (SD) Br]	(♀2/80*; 2.5%) statistically significant trend in the incidence (p=0.013; 0, 0, 0, 0, 1, 2)	Thyroid follicular adenocarcinoma (rat) Squamous cell carcinoma in the non-glandular stomach (rat)	Thyroid follicular adenocarcinoma and Squamous cell carcinoma in the non-glandular stomach	one 2-year F at 20000 ppm one 1-year F at 3000 ppm				aggregates within the urinary tract Probably low-moderate
	Thyroid follicular adenocarcinoma at 20000 ppm (♂4/80*; 5%) statistically significant positive trend in the incidence (p=0.042; 1, 0, 0, 1, 1, 4)	Yes**: Urinary bladder papilloma (rat) Squamous cell carcinoma in the non-glandular stomach (rat)	Yes**: Thyroid follicular adenocarcinoma and Squamous cell carcinoma in the non-glandular stomach	No generally 2-year M	males	MTD was not reached at the highest dose	oral, dietary	- Low
	Squamous cell carcinoma in the non-glandular stomach at 20000 ppm (♂2/80*; 2.5%) statistically significant positive trend in the incidence (p=0.042; 0, 0, 0, 0, 0, 2)	Yes**: Urinary bladder papilloma (rat) Thyroid follicular adenocarcinoma (rat)	Yes**: Thyroid follicular adenocarcinoma and Squamous cell carcinoma in the non-glandular stomach	Yes exclusive "early deaths"	males	MTD was not reached at the highest dose	oral, dietary	- Probably low
Mouse, Crl:CD-1(ICR)Br	none	none	none	-	-	-	oral, dietary	-

** Statistically significant difference from control group mean at the p-value 0.05 level*
*** - The increased incidence of tumours was considered as incidental*

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

Based on the available data, trinexapac-ethyl does not require classification for carcinogenicity according to Regulation (EC) No 1272/2008.

2.6.6 Summary of reproductive toxicity

2.6.6.1 Adverse effects on sexual function and fertility – generational studies

Table 57: Summary table of animal studies on adverse effects on sexual function and fertility – generational studies

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (Batch No; purity) , dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference
Two-generation reproduction toxicity study OECD 416 (1983) GLP Rat, Sprague-Dawley 30/sex/group The study is considered acceptable, despite some deviations	Trinexapac-ethyl, FL 882373/96.2% FL 892178/96.2% 0, 10, 1000, 10000, 20000 ppm Equal to 0, 0.7, 106.2, 662.9 and 1293.0 mg/kg bw/d (average of all values) Oral: diet Approximate number of dose weeks: F0 – 22-25; F1 – 20-23	**Parental** NOAEL: 106.2 mg/kg bw/d LOAEL: 662.9 mg/kg/d; ↓bw gain premating (F0 males Day 0-91: 9.6%; F1 males Day 0-84: 10.5%; F0 female Day 0-91: 14.8%); ↓FC premating (F1 males: average 5.9%) **Reproductive** (sexual function and fertility) NOAEL: ≥ 1293.0 mg/kg bw/d LOAEL: Not obtained. Did not cause adverse effects at highest dose tested.	Anonymous, 1991 B.6.6.1.1

↓- decrease compared to control; ↑- increase compared to control.

Table 58: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 59: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other studies relevant for toxicity on sexual function and fertility are available				

2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility – generational studies

In a 2-generation reproductive toxicity study there were no treatment related effects on reproduction parameters up to the highest dose tested (for more detailed data please refer to RAR Volume 3, section B.6.6.1.1). The following deviations from OECD 416 were reported in the first approval conclusion from the Addendum (January 2005) to the DAR (2003): A dosing error was made during F0 premating study days 21-28, when the dietary admixtures 1000 and 20000 mg/kg food were switched. Due to the feeding error, the premating period was extended for three weeks. The number of pregnant F1-rats in the control and 10 mg/kg food was 15, instead of the recommended 20. It is recommended that the males be sacrificed after the mating period, while the males in this study were sacrificed after a post-mating period of 6 weeks (20-25 weeks in all, depending on generation). Additionally, it was concluded that this old study do not appear to comply with the updated OECD 416 (2001): it did not include some endocrine disruption-related sensitive endpoints such as oestrous cyclicity, sperm parameters, the age of vaginal opening and preputial separation as well as spleen, pituitary, thyroid and adrenal glands weight for parental animals. The dose selection rationale was not reported and the selection of hundred fold dose interval between the lowest dose and next dose was not considered optimal with a view to demonstrating no-observed-adverse-effects level (NOAEL).

The NOAEL for reproduction toxicity was ≥20000 ppm (1293.0 mg/kg bw/day), the highest dose tested.

The NOAEL concerning systemic toxicity for parental animals in the 2-generation study was 1000 ppm (106.2 mg/kg bw/day) based on reduced bodyweight gain in the F0 and F1 generation males and in the F0 females as well as reduced food consumption in the F1 generation males. It was considered inappropriate to establish the LOAEL for parental toxicity at 1000 ppm (106.2 mg/kg bw/day) based on observed adverse effects (reduced bodyweight gain by 10.8% and average food consumption 5.8%) in the F1 males only. These findings without any other associated adverse effects were considered insufficiently relevant for setting the LOAEL. Furthermore, the same effects (reduced bodyweight gain and average food consumption) were not observed in the other rat studies at higher levels, i.e. repeated dose study at 346 mg/kg bw/day (13-week rat study) or even long term rat study at 392.7 mg/kg bw/day (104-week rat study).

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

The definition of reproductive toxicity in the CLP Regulation (Section 3.7.1.1 of Annex I) includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring.

Adverse effects on sexual function and fertility are defined (Annex I: 3.7.1.3) as any effect of a substance that has the potential to interfere with sexual function and fertility including, but not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive system.

For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. The following criteria for classification for adverse effects on sexual function and fertility are given in CLP regulation:

Classification in reproductive toxicity Category 1A is reserved for substances known to be reproductive toxicants in humans.

Classification in reproductive toxicity Category 1B is reserved for substances that are presumed to be reproductive toxicants in humans, and is largely based on data from animal studies where there is clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or not as a secondary non-specific consequence of other toxic effects.

Classification in reproductive toxicity Category 2 is reserved for substances that are suspected to be reproductive toxicants in humans, and where there is some evidence from experimental animals of an adverse effect on sexual function and fertility but where the evidence is not sufficiently convincing to place the substance in Category 1. The adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

The two-generation rat study has clearly shown that these criteria were not met as trinexapac-ethyl has no effects on sexual function and fertility at dietary concentrations of up to 20000 ppm (equal to 1293.0 mg/kg bw/day), at which parental toxicity was observed.

2.6.6.2 Adverse effects on development

Table 60: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL	Reference
Two-generation reproduction toxicity study OECD 416 (1983) GLP Rat, Sprague-Dawley 30/sex/group The study is considered acceptable, despite some deviations	Trinexapac-ethyl, FL 882373/96.2% FL 892178/96.2% 0, 10, 1000, 10000, 20000 ppm Equal to 0, 0.7, 106.2, 662.9 and 1293.0 mg/kg bw/d (average of all values) Oral: diet Approximate number of dose weeks: F0 – 22-25; F1 – 20-23	**Parental** NOAEL: 106.2 mg/kg bw/d LOAEL: 662.9 mg/kg/d; ↓bw gain premating (F0 males Day 0-91: 9.6%; F1 males Day 0-84: 10.5%; F0 female Day 0-91: 14.8%); ↓FC premating (F1 males: average 5.9%) **Offspring** NOAEL: 662.9 mg/kg bw/d LOAEL: 1293.0 mg/kg bw/d: ↓bw (both sexes F1 pups: ~20%; F2 pups: ~24%); decreased survival index (F1 sexes combined: Day 4-21; F2 female pups: Days 0-4)	Anonymous, 1991 B.6.6.1.1
Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP Rat, Sprague-Dawley, RAIf (SPF) hybrids of RII/1 × RII/2 24 females / dose group The study is considered acceptable	Trinexapac-ethyl, P.705002, 96.6% 0, 20, 200, 1000 mg/kg bw/d Days 6-15 of gestation, gavage	**Maternal:** NOEL: ≥ 1000 mg/kg bw/d LOAEL: Not obtained. Did not cause adverse effects at highest dose tested. **Developmental:** NOAEL: 200 mg/kg bw/d LOAEL: 1000 mg/kg bw/d: ↑ litter incidence of asymmetrically shaped sternebrae	Anonymous, 1988 B.6.6.2.1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL	Reference
Developmental toxicity (teratogenicity) study OECD 414 (1981) GLP Rabbit, New Zealand White 16-17 females / dose group The study is considered acceptable, despite deviation	Trinexapac-ethyl, P.705002, 96.6% 0, 10, 60, 360 mg/kg bw/d Days7-19 of pregnancy, gavage	**Maternal:** NOAEL: 60 mg/kg bw/d LOAEL: 360 mg/kg bw/d: ↑mortality, retarded body weight gain to Day 15 **Developmental:** NOAEL: 60 mg/kg bw/d LOAEL: 360 mg/kg bw/d: ↑ post-implantation loss; ↓ number of live foetuses	Anonymous, 1990 B.6.6.2.2

Table 61: Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 62: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
(Q)SAR analysis using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited)	Trinexapac-ethyl (CGA 163935)	The results of (Q)SAR analysis) on parent substance were submitted (16-03-2017) on the request by the RMS. The software compares the structures with known toxicity endpoints. When a structural alert is identified the programme assigns a probability to the expression of toxicity by the compound.	Trinexapac-ethyl did not trigger any Derek Nexus alert for ‘Developmental’ and/or ‘Teratogenicity’ endpoints. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.	Anonymous, 2017*

** - Syngenta has requested data confidentiality for these data pursuant to art.63 of Reg. (EC) 1107/2009*

2.6.6.2.1 Short summary and overall relevance of the provided information on adverse effects on development

This section is represented by one two-generation reproduction toxicity study in rat and two developmental toxicity studies in rat and rabbit. The results of two-generation reproduction (the offspring effects observed only) and developmental toxicity studies are summarised in Table 60.

The NOAEL concerning systemic toxicity for parental animals in the 2-generation study was 1000 ppm (106.2 mg/kg bw/d) based on reduced bodyweight gain in the F0 and F1 generation males and in the F0 females as well as reduced food consumption in the F1 generation males. The offspring effects were reduced body weight during and at the end of lactation period in two generations of both sexes (F1 pups: male 18.9%, female 20.5%, F2 pups: male 23.6%, female 24.1%) as well as reduced survival index (post-cull, days 4-21) in the offspring (F1 pups: sexes combined and pre-cull, days 0-4, F2 female pups) at the highest dose level. Hence, the NOAEL for offspring toxicity was 10000 ppm (662.9 mg/kg bw/day). The developmental effects noted in the two-generation reproduction toxicity study with trinexapac-ethyl do not provide sufficient evidence for classification for hazard category.

Two guideline-compliant (OECD 414) developmental toxicity studies performed in rats and in rabbit are available for trinexapac-ethyl (for more detailed data please refer to RAR Volume 3, section B.6.6.2.1 and B.6.6.2.2, respectively).

Worthy of notice that these old studies do not appear to comply with the updated OECD TG 414 (2001) as main differences were identified: the dose period covered solely the period of major organogenesis (i.e. days 6-15 in the rat and days 7-19 in the rabbit), groups were with fewer than 16 animals with implantation sites at necropsy (rabbit study) and six- to ten-fold intervals of doses were used, instead of recommended two- to four-fold intervals.

In the **developmental toxicity study conducted with rats** (Anonymous, 1988, B.6.6.2.1) no indication of maternal adverse toxicity could be detected at the international regulatory limit dose. Therefore, the maternal NOAELs were set at 1000 mg/kg bw/day, the highest dose tested.

According to this developmental toxicity study report group mean maternal body weights for the test article treated groups were comparable to the control group for the entire study period: there were no statistically significant differences. In the high dose group body weight gain was statistically significantly reduced for the period days 0 – 6 (8.1%) and for the entire study period (days 0 – 21) (5.6%). Hence, the magnitudes of mean body weight gain changes throughout the dosing period and at study termination didn't exceed 10%. In addition, corrected body weight and corrected body weight gain for all test article treated groups were comparable to the control group: there were no statistically significant changes. Thus mean body weight gain changes were not considered adverse. Mean food consumption for the high dose group was comparable to the control group: there were no statistically significant changes.

Regarding pregnancy data / uterine findings, the number of *corpora lutea* in the high dose group was statistically significantly decreased compared to the control group. The value for these finding (mean no. *corpora lutea* / dam) at the top dose level was just at the lower edge of historical control range given and was below the median of historical control data (HCD): median 17.5, min. 17.0, max. 19.1. Historical pregnancy data: 13 control groups with a total of 297 pregnant female were examined from September 1985 to April 1987. Due to the smaller number of *corpora lutea* in the high dose group, the number of implantation sites was also lower, however, to a lesser and statistically not significant extent. There were non-statistically significant pre-implantation losses and implantation efficiency in any of treated groups. The number of early resorptions was comparable for all experimental groups and late resorptions, dead or aborted foetuses were not detected in any of the groups. Since ovulation occurs before the start of dosing and there were not pre-implantation losses in the top dose group the decrease of number of *corpora lutea* was not considered relevant.

Caesarean section observations for all pregnant females

Observation	**Dose level (mg/kg bw/day)**			
	0 (control)	**20**	**200**	**1000**
Mated females assigned	24	24	24	24
Animals pregnant (%)	22 (91.7)	24 (100)	24 (100)	24 (100)
Animals not pregnant	2	0	0	0
Dams with live foetuses (% of dams)	22 (100)	24 (100)	24 (100)	24 (100)
Dams with all implants resorbed	0	0	0	0
Dams with any implants resorbed (% of dams)	12 (54.5)	7 (29.2)	11 (45.8)	14 (58.3)
Dams with aborted foetuses	0	0	0	0
Dams delivering prior to hysterectomy	0	0	0	0
Mean number corpora lutea/dam	19.1	18.7	18.7	17.00**
Mean number implantation sites/dam	16.6	15.9	16.1	15.7
Pre-implantation loss (%)	12.4	13.7	12.9	8.3
Implantation efficiency (%)	87.6	86.3	87.1	91.7
Mean number early resorptions/dam	0.6	0.4	0.8	0.7
Mean number late resorptions/dam	0	0	0	0
Number of dead foetuses	0	0	0	0
Number of aborted foetuses	0	0	0	0
Post-implantation loss (%)	4.0	2.8	4.6	4.9
Mean number live foetuses/dam	16.0	15.5	15.3	15.0
Mean number males/dam	7.8	7.3	7.5	7.0
Mean number females/dam	8.1	8.2	7.8	8.0
% males per group	49.0	47.0	48.8	46.0
Number of litters	22	24	24	24
Number live foetuses	351	372	367	359
Mean foetal body weight (g) males	5.7	5.6	5.7	5.7
Mean foetal body weight (g) females	5.3	5.4	5.3	5.4
Mean foetal body weight (g) combined	5.5	5.5	5.5	5.5

*** Statistically significant difference from control group mean, p<0.01*

Fetal effects: with respect to the overall incidence in fetal malformations and anomalies of dams treated with trinexapac-ethyl at doses of 20, 200, and 1000 mg/kg bw/d there was no statistically significant difference compared to the control group. Type and incidence of fetal visceral findings revealed no test article related effect, though one fetus of the high dose group showed hypoplasia of the left testicle. Skeletal examination of the foetuses revealed no malformations for the intermediate and high dose group.

However, there were some nonstatistically significant differences in type and incidence of skeletal anomalies in the test article treated groups compared to the control group and historical control groups: one fetus at the top dose showed fragmentary sternebra and an apparently dose dependent increase of asymmetrically shaped sternebrae was observed. Value for the latest finding (asymmetrically shaped sternebrae, litter incidence, %) was outside the historical control range (mean + 1SDV) for the laboratory. Historical incidence of skeletal anomalies: 10 controls with a total of 234 pregnant females were examined from September 1985 to April 1987.

The incidences of still absent ossification were statistically significantly (CHI-square test) increased for several cervical vertebral centers (CVC 3, CVC 4, CVC 5) of the low and high doses group, however, they showed no dose dependency, the high dose group values were within the HCD and therefore these findings were considered not treatment related.

Incidence of foetal skeletal anomalies (excerpt)

	group 1	group 2	group 3	group 4
dose (mg/kg)	0	20	200	1000
litters examined	22	24	24	24
fetuses examined	234	248	245	239
asymmetrically shaped sternebrae				
affected fetuses	2	4	5[3]	8[4]
(%)	0.9	1.6	2.0	3.3
affected litters	2	4	4	7
(%)	9.1	16.7	16.7	29.2
fragmentary sternebra				
affected fetuses	0	0	0	1
(%)	-	-	-	0.4
affected litters	0	0	0	1
(%)	-	-	-	4.2

[3] – One fetus also showed wide fontanel and bipartite sternebra
[4] - One fetus also showed fragmentary sternebra and other also showed fused sternebra

Historical incidence of foetal skeletal anomalies (excerpt)

	Litter Incidence N	Litter Incidence %	Litter Incidence SD	Fetal Incidence N	Fetal Incidence %	Fetal Incidence SD
No. Evaluated	234			2093		
asym. shaped						
STE1	7	2.96	6.53	7	0.33	0.75
STE2	2	0.85	1.79	2	0.09	0.19
STE3	2	0.87	1.83	2	0.13	0.28
STE4	6	2.61	3.11	7	0.35	0.41
STE5	25	10.74	6.33	30	1.39	0.92
STE6	6	2.65	6.05	11	0.53	1.26
STE (total)	35	15.08	11.57	48	2.28	2.09

Due to a lack of statistically significant differences in incidence of skeletal anomalies (asymmetrically shaped sternebrae, litter incidence, %) in the highest dose group compared to the control group at first was considered by RMS LT treatment related but not adverse. This was based on following considerations: *Asymmetric sternebra* in rats as well as in others species should be considered as a "grey zone anomaly" according to current state of art, i.e. the updated harmonized nomenclature for developmental toxicology, based on the revised IFTS terminology (Makris et al. 2009), (last update October 2012). It means that this anomaly does not fit readily into one of the two categories (malformation or variation). Litter incidence of *asymmetrically shaped sternebrae* (29.2%) at the top dose was outside the historical control range (15.08±11.57%) for the laboratory. Historical incidence of skeletal anomalies: 10 controls with a total of 234 pregnant females were examined from September 1985 to April 1987. There was a non-statistically significant increase in the litter incidence of asymmetrically shaped sternebrae in the highest dose group compared to the control group, therefore it was not considered to be adverse. The applicant has submitted an additional HCD from separate 12 developmental toxicity studies conducted at the same laboratory, covering several years before and after this study (1987-1993). However, total data of 297 pregnant females have not been combined in a single package and SD/ranges have not been reported. Based on the additional HCD it can be concluded that litter incidence of asymmetrical sternebra at the high treatment group is below the incidence mean for this finding only in three studies from twelve. *Vertebra cervical centrum incomplete ossification* should be considered as a "variation", whereas *cervical centrum unossified* should be considered as a "grey zone anomaly" according to the harmonized nomenclature for developmental toxicology mentioned above. There was no difference in the litter incidences of still absent ossification for several cervical vertebral centers (CVC 3, CVC 4, CVC 5), whereas the foetuses incidences were statistically significantly (CHI-square test) increased of the low and high doses group, however, they showed no dose dependency. Furthermore, the high dose group values were within the HCD range and therefore these findings were considered not treatment related. Therefore, asymmetrically shaped sternebrae and still absent ossification should not be considered together.

However, during the peer review meeting (PPR 170, 11 – 14 December 2017) experts discussed if observed asymmetrically shaped sternebrae should be taken into consideration when setting NOAEL for this study. Experts quoted ECETOC Guidance (2002) and Moore et al. (2013) where asymmetric sternebra considered as an anomaly or malformation of high concern. The incidences were above the HCD (1985-1987) and equal to maximum observed in the HCD (1987-1993) at the top dose level. Finally, experts agreed to set NOAEL at the level of 200 mg/kg bw per day and RMS LT supported suggested NOAEL.

Experts discussed a need to propose classification of the substance based on the observed effect on asymmetrically shaped sternebrae and discussed the adversity of observed effects. According with the description of the effects in the study report it is not clear if the observed effect is malformation or delayed ossification. Some experts questioned if the re-evaluation of data is possible by the pathologist. Additionally, there was an effect on body weight gain in dams observed (6%), treatment-related, but not adverse and it was suggested that this might have affected the litters in which asymmetrically shaped sternebrae were observed, but no evaluation of correlation was conducted.

No consensus was achieved by experts regarding the proposal for classification of the substance based on the observed findings of asymmetrically shaped sternebrae. The RMS LT did not support classification and labelling. Thus in EFSA conclusion no proposition for classification will be suggested as proposed by RMS.

References quoted during the meeting:

- *Crit Rev Toxicol. 2013 Nov;43(10):850-91. doi: 10.3109/10408444.2013.854734.Guidance on classification for reproductive toxicity under the globally harmonized system of classification and labelling of chemicals (GHS). Moore NP, Boogaard PJ, Bremer S, Buesen R, Edwards J, Fraysse B, Hallmark N, Hemming H, Langrand-Lerche C, McKee RH, Meisters ML, Parsons P, Politano V, Reader S, Ridgway P, Hennes C.*
- *ECETOC 2002. Guidance on Evaluation of Reproductive Toxicity Data. Monograph N 31. ISSN-0773-6347-31.*

In the second **developmental toxicity study in rabbits** (Anonymous, 1990, B.6.6.2.2) the following deviation from OECD 414 was reported: the females were exposed to the test substance on gestation days 7-19, instead of the recommended 6-18. The study report was checked for compliance with OECD 414 (Prenatal Developmental Toxicity Study) (adopted 22nd January 2001)) and it was concluded that the study does not appear to comply fully with the updated OECD guideline. The major differences between the modern guideline requirements and the trinexapac-ethyl rabbit study were: there were less than 20 female animals per group with implantation sites at necropsy and six-fold interval was used, instead of recommended two- to four-fold intervals for setting the descending dose levels.

Regarding rabbit developmental study conducted on rabbits, the maternal NOAEL was set at 60 mg/kg bw/day, based on increased mortalities and retarded body weight gain to Day 15 at 360 mg/kg bw/day dose. The two mortalities at 360 mg/kg/d were considered to be associated with treatment and the first death occurred on day 13 (6 days after dosing). It is noteworthy that there were 4/6 and 1/6 mortalities in a preliminary study at 800 mg/kg bw/day and at 400 mg/kg bw/day, respectively. The mortalities were attributed to substance irritation of the stomach mucosa as the animals had haemorrhagic depressions in the stomach. Body weight gain of animals at 360 mg/kg/d dose was retarded relative to control, low and mid dose groups to Day 15: 13 females from 14 and 11 from 14 had reduced body weight gain on Day 9 and 11, respectively. It should be noted that two females in 360 mg/kg/d dose group showed depressed gains/loss throughout: one female did not recover and one female had regained the weight loss on Day 29. However, there were no statistically significant and/or dose related differences in mean body weights and food consumption during treatment period (gestation days 7-19) and/or during gestation in all dose groups compared to controls. It should be noted that information on corrected maternal body weight and corrected maternal body weight gain for all groups is not available for this study.

Regarding developmental effects there was a statistically significant decrease in the number of live foetuses and increase in pre-implantation loss (%) and post-implantation loss (%) in the top dose group compared to controls. However, there were no statistically significant changes in non-percentage of pre-implantation and post-implantation losses. The magnitude of the increases in post-implantation loss in the top dose group was slightly higher than in pre-implantation loss compared to controls. Additionally, pre-implantation (80%) and post-implantation (80%) losses were observed in higher number of females compared to controls, 50% and 60%, respectively. However, a relationship of decrease in the number of live foetuses to treatment cannot be conclusively established on the basis of the information provided since the treatment started after unequal pre-implantation loss. Historical control data was not available but would be useful and appropriate for interpreting study findings. On the basis of the insufficient information to conclude, differences in litter size were considered attributable to treatment. Data are summarised in the table below:

Caesarean section observations

Observation	**Dose level (mg/kg bw/day)**			
	0 (control)	**10**	**60**	**360**
Animals Assigned (Mated)	16	16	17	17
Animals Pregnant	14	16	16	16
Animals Non pregnant	2	0	1	1
Animals Aborted	1	0	1	0
Animals killed intercurrent	0	0	1	2[(a)]
Animals Totally resorbed	1	0	0	0
Total Litters (viable)	12	16	14	14
Corpora Lutea/Dam	10.5	10.9	10.9	10.2
Pre-implantation Loss/Dam	1.7	1.7	1.8	2.6 ↑52.9%
Implantations/Dam	8.8	9.2	9.1	7.6 ↓13.6%
Pre-implantation Loss (%)	14.3	16.5	16.2	24.3* ↑10.0%
Live Foetuses/Dam	7.7	8.4	7.0	5.7* ↓26.0%
Total embryonic deaths/Dam	1.2	0.8	2.1	1.9 ↑58.3%
Early embryonic deaths/Dam	0.6	0.2	1.4	1.0
Late embryonic deaths/Dam	0.6	0.6	0.7	0.9
Post-implantation Loss (%)	13.2	8.1	21.4	24.8* ↑11.6%
Litter Weight (g)	332.0	360.7	320.5	255.7 ↓23.0%
Mean Foetal Weight (g)	44.4	43.8	47.0	45.2
Sex Ratio (% Males per litter)	40.9	56.9	53.5	56.7

[(a)] includes 1 animal which aborted prior to terminal sacrifice on day 29

** Statistically significant different trend from control group mean, $p<0.05$ (Jonckheere "J" statistic)*

↓↑% - compared to control

Hence, the developmental NOAEL was 60 mg/kg bw/day, based on increased post-implantation loss and the decrease in the number of live foetuses at 360 mg/kg bw/day. No teratogenic effects were observed in rabbit. The effects noted in the rabbit developmental toxicity study with trinexapac-ethyl were not sufficient to trigger a proposal for classification for hazard category.

It should be noted that based on (Q)SAR analysis (using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited) submitted, trinexapac-ethyl did not trigger in any Derek Nexus structural alert for developmental toxicity and/or teratogenicity. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

The definition of reproductive toxicity in the CLP Regulation (Section 3.7.1. of Annex I) includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring.

Adverse effects on development of the offspring (Annex I: 3.7.1.4) includes any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or post-natal, to the time of sexual maturation. As classification for developmental toxicity is primarily intended to provide a hazard warning for pregnant women and for men and women of reproductive capacity, for pragmatic purposes, classification for developmental toxicity is essentially intended to encompass adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. The following criteria for classification for adverse effects on development are given in CLP regulation:
Classification in reproductive toxicity Category 1A is reserved for substances known to be reproductive toxicants in humans.
Classification in reproductive toxicity Category 1B is reserved for substances that are presumed to be developmental toxicants in humans, and is largely based on data from animal studies where there is clear evidence of an adverse effect on development in the absence of other toxic effects, or not occur as a secondary non-specific consequence of other toxic effects.
Classification in reproductive toxicity Category 2 is reserved for substances that are suspected to be reproductive toxicants in humans, and where there is some evidence from experimental animals of an adverse effect on development but where the evidence is not sufficiently convincing to place the substance in Category 1. The adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

The offspring effects noted in the two-generation reproduction toxicity study were reduced body weight during and at the end of lactation period in two generations of both sexes, reduced survival index in the offspring at the highest dose level only.

The developmental effects noted in the rabbit developmental toxicity study with trinexapac-ethyl were increased post-implantation loss and the decrease in the number of live foetuses at the highest dose level only.

The developmental effect noted in the rat developmental toxicity study with trinexapac-ethyl was increase in the litter incidence of asymmetrically shaped sternebrae at the highest dose level only. This skeletal anomaly (asymmetrically shaped sternebrae) was considered as a “grey zone anomaly” according to current state of art and it means that this anomaly does not fit readily into one of the two categories (malformation or variation).

All these findings are not considered toxicological significant effect and/or changes in the proportions of foetal variants of high concern based on weight of evidence approach and in consideration of the important factors in Annex I Sections 3.7.2.3.3. and 3.7.2.4.2. According to CLP, Annex I, Section 3.7.2.3.3 “*If, in some* *reproductive toxicity studies in experimental animals the only effects recorded are considered to be of low or minimal toxicological significance, classification may not necessarily be the outcome. These effects include small changes …in the incidence of spontaneous defects in the foetus, small changes in the proportions of common foetal variants such as are observed in skeletal examinations, or in foetal weights, or small differences in postnatal developmental assessments*" and Section 3.7.2.4.2., "…*classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations embryo/foetal lethality, significant post-natal functional deficiencies*".

Hence, adverse effects on development noted in the two-generation reproduction toxicity study in rat and the developmental toxicity studies in rats and rabbits were not sufficient to trigger a proposal for classification for this hazard category.

2.6.6.3 Adverse effects on or via lactation

Table 63: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Two-generation reproduction toxicity study OECD 416 (1983) GLP Rat, Sprague-Dawley 30/sex/group The study is considered acceptable, despite some deviations	Trinexapac-ethyl, FL 882373, 96.2% FL 892178, 96.2% 0, 10, 1000, 10000, 20000 ppm Equal to 0, 0.7, 106.2, 662.9 and 1293.0 mg/kg bw/d (average of all values) Oral: diet Approximate number of dose weeks: F0 – 22-25; F1 – 20-23	**Parental** NOAEL: 106.2 mg/kg bw/d LOAEL: 662.9 mg/kg/d; ↓bw gain premating (F0 males Day 0-91: 9.6%; F1 males Day 0-84: 10.5%; F0 female Day 0-91: 14.8%); ↓FC premating (F1 males: average 5.9%) **Offspring** NOAEL: 662.9 mg/kg bw/d LOAEL: 1293.0 mg/kg bw/d: ↓bw (both sexes F1 pups: ~20%; F2 pups: ~24%); decreased survival index (F1 sexes combined: Day 4-21; F2 female pups: Days 0-4) **Reproductive** NOAEL: ≥ 1293.0 mg/kg bw/d LOAEL: Not obtained. Did not cause adverse effects at highest dose tested.	Anonymous, 1991 B.6.6.1.1

Table 64: Summary table of human data on effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 65: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other studies relevant for effects on or via lactation are available				

2.6.6.3.1 Short summary and overall relevance of the provided information on effects on or via lactation

There is no evidence from the two-generation reproductive study (Anonymous, 1991 (B.6.6.1.1)) for specific effects of trinexapac-ethyl treatment on lactation or via lactation on offspring. Offspring effects in this study were limited to reduced body weight during and at the end of lactation period (F1 pups: male 18.9%, female 20.5%, F2 pups: male 23.6%, female 24.1%) as well as reduced survival index (post-cull, days 4-21) in F1 pups (sexes combined) and (pre-cull, days 0-4) in F2 female pups at the highest dose level. However, this concentration of 20000 ppm led to a reduced body weight in the F0 and F1 generation females (F0: premating 16.6%, gestation 14.2%, 7-day lactation 14.1%; F1: premating 16.0%, gestation 10.9%, 7-day lactation 14.2%). Therefore, offspring effects were associated with reduced maternal body weight and are not considered to be a direct effect of trinexapac-ethyl exposure via lactation.

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

In accordance with the CLP Regulation (Section 3.7.1.2 of Annex I), for the purpose of classification the hazard class Reproductive Toxicity is differentiated into adverse effects on sexual function and fertility or on development as well as into effects on or via lactation.

Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk (Section 3.7.2, Table 3.7.1(b) of Annex I of the CLP Regulation).

Adverse effects on or via lactation are included under reproductive toxicity, but for classification purposes such effects are treated separately (section 3.7.1.5 of Annex I of the CLP Regulation). The classification of a substance is derived from the hazard categories in the following order of precedence: Category 1A, Category 1B, Category 2 and the additional Category for effects on or via lactation. Classification in the additional category for effects on or via lactation will be considered irrespective of a classification into Category 1A, Category 1B or Category 2.

The two-generation reproductive study has shown that these criteria were not met as limited offspring effects (reduced body weight and reduced survival index) at the highest dose level were associated with reduced maternal body weight and are not considered to be a direct effect of trinexapac-ethyl exposure via lactation.

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

No effects on sexual function or fertility in the two-generation rat study were observed which are considered relevant for potential classification of trinexapac-ethyl as reproductive toxicant according to Regulation (EC) No 1272/2008.

The effects noted in the rat and rabbit developmental toxicity studies with trinexapac-ethyl were not sufficient to trigger a proposal for classification for this hazard category according to Regulation (EC) No 1272/2008.

No classification of trinexapac-ethyl in the additional category for effects on or via lactation is proposed.

2.6.7 Summary of neurotoxicity

Trinexapac-ethyl has been tested in short term and chronic toxicological studies at a wide range of dose levels in dog, rat and mouse. Brain vacuolation was seen only in dogs and they were found to be the most susceptible species with regard to the cerebral vacuolation effects. The cerebral vacuolation was treatment-related, age-dependent and dosage-dependent by comparison of feeding studies in dogs (for more detailed data please refer to RAR Volume 3, section B.6.3.2.3.). In the 13-week study, only one male of eight dogs was affected at the high level (30000 ppm, equal to 930 mg/kg bw/day).

A treatment-related and dose dependent vacuolation of glial cells of forebrain/midbrain regions was seen at 10000 ppm (365.7 and 357.1 mg/kg bw/day for males and females, respectively) and 20000 ppm (726.7 and 783.8 mg/kg bw/day for males and females, respectively) in a 52-wk oral dog toxicity study (please refer to section 2.6.3.1; Table 46; Anonymous, 1992 (B.6.3.2.3)). The incidences were statistically significantly increased only at 20000 ppm: all animals showed this lesion. The compound-related vacuoles were generally larger in size and more closely clumped than the artefactual vacuoles from control and other dogs. The two supplementary reports with additional information regarding effects of the trinexapac-ethyl on brain were given for the renewal of approval of the active substance (*Persohn E, 1999* (B.6.3.2.3.1.) and *Krinke G., 1994* (B.6.3.2.3.3.)). The topographical distribution of the lesion involved three forebrain and two midbrain regions at 20000 ppm as well as one forebrain region at 10000 ppm in both sexes. The vacuolation was mostly located in the white brain matter, in the zone of transition between the white and the grey brain matter. The lesion was confined to a bilateral - symmetrical swelling of oligodendroglial and astrocytic cells, without progression to more advanced or more extensive damage of the nervous tissue. Nerve cells were not vacuolated. The cerebral vacuolation in dogs was not associated with any neurodegenerative/inflammatory histopathological changes or overt neurological signs. The mild, probably reversible effect on glia cells was probably induced by an interference with energy (glucose) metabolism and/or synthesis of nucleic acids and proteins. In the absence of mechanistic studies and/or any human data, the cerebral vacuolation was considered as relevant for humans.

Rat acute and subchronic 13 week neurotoxicity studies were conducted for US EPA regulatory requirements and are thus now presented as further information within the Renewal Process for the Renewal Assessment Report. The neurotoxicity studies presented were performed in compliance with GLP standards. No neurotoxic effects were observed in the acute or subchronic rat neurotoxicity studies. A study on delayed neurotoxicity being only required for organophosphorus or carbamate compounds was not considered warranted as neither trinexapac-ethyl nor any of the metabolites are belonging to these chemical classes.

Table 66: Summary table of animal studies on neurotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance(Batch No; purity), dose levels duration of exposure	Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL	Reference
Acute neurotoxicity study OECD 424 (1997) GLP Rat, Crl:CD(SD) 10/sex/dose The study is considered acceptable.	Trinexapac-ethyl, SMO8E551, 95.8% 0, 500, 1000, 2000 mg/kg bw/d Single oral dose, gavage	Neurotoxicity NOAEL: ≥ 2000 mg/kg bw/d Neurotoxicity LOAEL: Not obtained. No signs of neurotoxicity observed at highest dose tested Systemic NOAEL: ≥ 2000 mg/kg bw/d Systemic LOAEL: Not obtained. Did not cause adverse effects at highest dose tested	Anonymous, 2012 B.6.7.1.1
Subchronic (13 week) dietary neurotoxicity study OECD 424 (1997) GLP Rat, Crl:CD(SD) 12/sex/dose The study is considered acceptable.	Trinexapac-ethyl, SMO8E551, 95.8% 0, 3750, 7500, 15000 ppm Equal to 0, 233, 463, 948 mg/kg bw/d for males and 0, 294, 588, 1171 mg/kg bw/d for females 13 weeks oral, dietary	Neurotoxicity NOAEL: ≥ 948 mg/kg bw/d Neurotoxicity LOAEL: Not obtained. No signs of neurotoxicity observed at highest dose tested. Systemic NOAEL: ≥ 948 mg/kg bw/d Systemic LOAEL: Not obtained. Did not cause adverse effects at highest dose tested.	Anonymous, 2012a B.6.7.1.2

In the acute neurotoxicity study groups of 10 male and 10 female Crl:CD(SD) rats were given single oral doses of 0 , 500, 1000 and 2000 mg/kg bw trinexapac-ethyl by gavage. The observed initial effects on body weight gain and food consumption in males as well as the initial differences in total motor activity counts in females at 2000 mg/kg bw were considered to be treatment related but not to be of toxicological significance due to the small magnitude, transient / isolated nature and limitation by one sex. No treatment-related findings were noted during the FOB investigation. Brain weight and dimensions determination and neuropathology microscopic examination did not reveal any neuropathological, treatment-related findings up to 2000 mg/kg bw. The NOAEL for neurotoxicity and systemic toxicity following a single oral dose was 2000 mg/kg bw for both sexes.

In the subchronic neurotoxicity study groups of 12 male and 12 female Crl:CD(SD) rats were given diets containing 0, 3750, 7500 or 15000 ppm trinexapac-ethyl for 13 weeks (corresponding to 0, 233, 463 and 948 mg/kg bw/day in males, and 0, 294, 588 and 1171 mg/kg bw/day in females). The observed initial effects on body weight gain and food consumption in females at 15000 ppm were considered to be treatment related but not to be adverse due to the transient nature, limitation by one sex and in absent any other accompanying effects. An increased incidence of a more energetic response to tail pinch and a corresponding statistically significant decreased incidence of the animal slowly turning and walking away from a tail pinch were noted for the 15000 ppm males during study week 12. These test substance-related findings were not considered to be adverse as they were only observed during the last interval and occurred in the absent of effects on any other related endpoints. No other treatment-related findings were noted during the FOB investigation. Locomotor activity, ophthalmology, brain weight and dimensions determination as well as neuropathology microscopic examination did not reveal any neuropathological, treatment-related findings up to 15000 ppm. The NOAEL for neurotoxicity systemic toxicity following treatment with trinexapac-ethyl in the diet for 13 weeks was 15000 ppm (948 mg/kg bw/day) for both sexes.

It should be noted that based on (Q)SAR analysis (using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited) submitted, trinexapac-ethyl did not trigger in any Derek Nexus structural alert for 'Neurotoxicity' and/or 'Cholinesterase inhibition' endpoints. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.

2.6.8 Summary of other toxicological studies

2.6.8.1 Toxicity studies of metabolites and impurities

Metabolites. The ADME shows that the major metabolite of trinexapac-ethyl (CGA163935) after oral administration in rats is metabolite CGA179500 (*4-[cyclopropyl(hydroxy)methylidene]-3,5-dioxocyclohexane-1-carboxylic acid*, other IUPAC names: trinexapac and 4-(cyclopropyl-hydroxy-methylene)-3,5-dioxo-cyclohexanecarboxylic acid; CAS No 143294-89-7, both in urine and faeces. The active substance is extensively metabolised by mainly hydrolysis: forty-eight hours after low dose administration, 92% of the cumulative urinary radiolabel consists of this metabolite.

Information on so-called dietary metabolites and groundwater metabolites is not relevant for the CLP proposal for the active substance. It should be noted that dietary metabolites were defined by RMS in this case as metabolites to which humans or livestock were exposed, i.e. in crops, in commodities upon processing, in food of animal origin or in feed, respectively, based on residue section data. At the expert meeting (PPR 170, 11 – 14 December 2017), the data gap was proposed to address the repeated exposure toxicity (available 90-day rat study to JMPR) and updated literature search of the metabolite CGA224439. The toxicity studies with these metabolites, the summaries and conclusions on toxicity of so-called dietary metabolites, or potential dietary metabolites are presented RAR Volume 3, section B.6.8.1.

Impurities. The issue the potential toxicity of impurities in the technical specification was evaluated at length in the confidential part (for more detailed data please refer to Volume 4 – Annex C - Confidential information, Syngenta, Section C.1.4. and Section C.1.5.). The impurity profile remains confidential, therefore this information is presented in the confidential part only. It should be noted that from a toxicological point of view the impurity (1*RS*)-ethyl 3-hydroxy-5-oxocyclohex-3-ene-1-carboxylate (CGA158377) and toluene are considered relevant based on their hazard (skin sensitisation and reproductive toxicity respectively). Additionally, further data are needed to confirm the purity content of batches used in toxicity studies and because further data would be needed to exclude the relevance of some others impurities. These impurities were either not tested at sufficiently high level or not detected in the technical material used in the relevant studies. Therefore, the toxicological relevance of these impurities cannot be concluded on the basis of the available data. Hence, a conclusion on whether the batches used in the toxicity studies submitted by Syngenta Crop Protection

AG was representative of the proposed technical specification could not be drawn lessding to a critical area of concern.

2.6.8.2 Supplementary studies on the active substance

28-Day immunotoxicity feeding study in mice and a review concerning immunotoxicity potential

An immunotoxicity study and a detailed review of parameters related to immune function with the existing toxicity database for trinexapac-ethyl were submitted.

A detailed review of parameters related to immune function has been conducted on the existing toxicity database for trinexapac-ethyl (for more detailed data please refer to RAR Volume 3, section B.6.8.2.2.). Repeat-dose studies in rats, mice and dogs were reviewed for any treatment-related changes in a variety of indicators of potential immunotoxicity including white blood cell counts and /or differential counts, globulin levels in plasma, organ weights (spleen, thymus and adrenals), and microscopic findings (bone marrow, lymph nodes, spleen, thymus and adrenals). The review of the toxicology database for trinexapac-ethyl has shown no evidence of adverse effects on the immune system in rats, mice or dogs. Thymus atrophy, alterations in haematology parameters (white blood cell counts and/or differential counts) and thymus weights in 90 day dog study were considered to be a secondary effect related to a primary non-immunotoxic outcome, i.e. the presence of overt general toxicity.

In addition, trinexapac-ethyl does not belong to a class of chemicals (e.g., the organotins, heavy metals, or halogenated aromatic hydrocarbons) that would be expected to be immunotoxic. There was no evidence from the literature that trinexapac-ethyl was immunotoxic and no clinical case reports or poisoning incidences were known indicating an immunotoxic potential.

An immunotoxicity study has been conducted according to US EPA OPPTS 870.7800 (1998) to fulfil data requirements of the US-EPA (for more detailed data please refer to RAR Volume 3, section B.6.8.2.1.).

Table 67: Summary table of animal studies on immunotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance(Batch No; purity), dose levels duration of exposure	Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL	Reference
28-Day immunotoxicity feeding study Immunotoxicity US EPA OPPTS 870.7800 (1998) GLP Mouse (female), B6C3F1 10/females/group and subsets AFC/NKC The study is	Trinexapac-ethyl, SMO5D180, 96.6% 0, 500, 2000, 5000 ppm Equal to average 0, 160.2, 613.7, 1630.5 mg/kg bw/d 28-days oral, dietary	Immunotoxicity NOAEL: ≥ 1530.5 mg/kg bw/d. Immunotoxicity LOAEL: Not obtained. No signs of immunotoxicity (the humoral and innate immune response) observed at highest dose tested. Systemic NOAEL: ≥ 1530.5 mg/kg bw/d Systemic LOAEL: Not obtained. Did not cause adverse effects at highest dose tested.	Anonymous, 2011 B.6.8.2.1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance(Batch No; purity), dose levels duration of exposure	Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL	Reference
considered acceptable.			

The study conducted with trinexapac-ethyl in female mice did not reveal any signs of immunotoxicity when administered via the diet over a period of 28 days. The results of the study up to 1530.5 mg/kg bw/day, the highest tested dose, showed that treatment did not cause any effects on the humoral immune response as assessed by T cell dependent antibody to sheep red blood cells or effects on spleen weights (the splenic Antibody-Forming Cell (AFC) assay). In addition, the treatment did not cause any effects on the innate immune response as assessed by natural killer cell activity or effects on spleen as well as thymus weights (the Natural Killer Cell (NKC) assay).

No clinical signs of systemic toxicity were observed in any dose groups: there were no adverse effects on body weight, body weight changes or nutritional parameters in female rats fed 0, 500, 2000, and 5000 ppm trinexapac-ethyl at termination and throughout the study.

The NOAEL for immunotoxicity and systemic toxicity under the conditions of the present study in female mice was ≥ 5000 ppm (equal to approximately 1530.5 mg/kg bw/day), the highest concentration tested.

It should be noted that based on (Q)SAR analysis (using DEREK NEXUS version 5.0.2 (NEXUS 2.1.1 LHASA Limited) submitted, trinexapac-ethyl did not trigger in any Derek Nexus structural alert for 'Cumulative effect on white cell count and immunology' endpoint. For more detailed data please refer to Volume 4 Syngenta, section C.1.4.2.2.

2.6.8.3 Endocrine disrupting properties

The notifier has reviewed and summarised all of the relevant available data, including open scientific literature, on trinexapac-ethyl for potential for endocrine disruption in mammalian species using a weight of the evidence approach proposed by the European Chemical Industry Council (CEFIC) Endocrine Modulators Steering Group (EMSG), structured according to the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters. Trinexapac-ethyl has been extensively tested, with the relevant data from the regulatory studies and open scientific literature covering a wide range of study types *in vitro* and *in vivo*. These data fall into levels 2, 4 and 5 of the OECD Conceptual Framework. Following a comprehensive review of all of these available data, only a single effect of potential relevance was identified by the notifier: statistically significantly lower group mean absolute and relative (to body weight) uterus weights were noted for females administered ≥ 1000 ppm in level 4 assay, 52-week feeding study in dogs (please refer to section 2.6.3.1; Table 46; Anonymous, 1992 (B.6.3.2.3)). This isolated finding was not considered by the notifier as reflection of effect on the endocrine system due to a number of methodological and reporting deficiencies of the supplementary report (Krinke G., Mahrou A., 1999 (B.6.3.2.3.2.)), lack of any histopathological lesions, any effects on the other organs of the female reproductive system in this and in 13-week study as well as luck of effects on the female endocrine system in any other study, including a two generation reproductive toxicity study. In addition, trinexapac-ethyl was demonstrated to not interact with isolated components of the endocrine system, including oestrogen receptors, *in vitro* according to the notifier.

As part of the United States Environmental Protection Agency (US EPA) ToxCastTM program, trinexapac-ethyl was evaluated for potential effects in an extensive battery of *in vitro* assays aimed at identification of potential endocrine activity according to the notifier. Trinexapac-ethyl was negative in all of these assays, providing comprehensive evidence that trinexapac-ethyl does not interact with isolated components of the endocrine system according to this information of ToxCast.

Toxicological studies on endocrine disrupting potential of trinexapac-ethyl identified in the literature were very limited. One in vitro assay (OECD CF Level 2) of high relevance determined the estrogenic potential of trinexapac-ethyl using MCF-7 cells, which proliferate in response to activators of the oestrogen receptor. Since trinexapac-ethyl gave an RPE of <10% (relative [to the response elicited by the positive control 17β-estradiol] proliferation effect), was therefore considered negative for estrogenic activity.

Following evaluation of each of the relevant studies individually and a subsequent weight of evidence evaluation, it was concluded by the notifier that trinexapac-ethyl cannot be considered an endocrine disrupter as defined by WHO/IPCS (2002).

The RMS agrees that the most studies available do not give any clear indications of an endocrine potential of trinexapac-ethyl. No specific studies were submitted for the evaluation of endocrine disruption properties of trinexapac-ethyl (levels 2 and 3). Trinexapac-ethyl has been extensively tested in mammalian species, including repeat dose, developmental and reproductive toxicity studies that fall into high levels (4 and 5) of the OECD Conceptual Framework. However, it should be noted that there were differences in the interpretations of some findings (for more detailed data please refer to RAR Volume 3, section B.6.3.2.3. and section B.6.8.3.). The concern for endocrine disrupting potential was raised from the supplementary report with additional information regarding the trinexapac-ethyl 1 year dog study.

The supplementary report with additional information (Krinke G., Mahrous A., 1999 (B.6.3.2.3.2.)) regarding the trinexapac-ethyl 1 year dog study (Anonymous, 1992 (B.6.3.2.3)) was given for the renewal of approval of the active substance: the ovaries, uterus, vagina and mammary gland were retrieved from the archives and evaluated by light microscopy to determine at which stage of the oestrus cycle, these female dogs were at termination. No histopathological effects were seen in the uterus at any dose in this report. Data on the oestrus cycle of the individual test females has demonstrated dose dependent pattern: all females at the two highest doses were in the middle/late oestrus cycle stage, whereas fewer females at low doses were in the same oestrus cycle stage. Absolute uterus weight changes were consistent with the physiological changes of uterus occurring at the different stages of the oestrus cycles: the large size of the uterus was determined by glandular proliferation with or without secretion and *vice versa*. Thus reduction in mean absolute uterus weight at the two highest doses could be explained by a physiological change of uterus occurring at the middle/late metoestrus cycle stage: regression/inactivity of glands and/or no glandular proliferation at these doses were established.

The oestrus cycle comprises the recurring physiologic changes for which the hormonal status of the females is of critical importance. Since a robust evaluation of oestrus cyclicity and hormone analysis was not carried out as well as a number of methodological deficiencies were identified in this specific supplementary report (e.g. the unclear origin of the classification scheme, only the histology of the uterus reported, the use of a single time point and the low number of animals), it is difficult to assess the biological relevance of the results. However, an adverse effect of trinexapac-ethyl on the oestrus cycle via a hormonally mediated mechanism at the two highest doses cannot be ruled out and therefore this effect was considered toxicologically relevant and the LOAEL for these findings was set at 10000 ppm (equal to 357.1 mg/kg bw/day for female).

In addition, higher level assay, a two-generation reproductive toxicity test in rats, did not include some endocrine disruption-related sensitive endpoints such as oestrous cyclicity, sperm parameters, the age of vaginal opening and preputial separation as well as spleen, pituitary, thyroid and adrenal glands weight for parental animals. It was concluded that this old study do not appear to comply with the updated OECD 416 (2001). Older reproductive toxicity studies that lack sensitive endpoints (e.g. onset of puberty) cannot fully exclude the possibility that chemicals testing negative may still be EDs.

The RMS noted that concentration of the maximal tolerated dose 20000 ppm led to an increased relative organ weight in F0 parental females (ovarian 23.1%) and F1 parental males and parental females (testes 17.5%, ovarian 32.0%). No adverse effects on any reproductive parameters (F0 and F1) investigated were observed in this study. Though no histopathologic alterations were observed in the reproductive organs, evaluation of sperm parameters, oestrous cycle length and normality was not performed. The NOAEL for parental toxicity was set at 1000 ppm based on reduced bodyweight gain and reduced food consumption.

It should be noted that according to the GD on Standardized Test Guidelines for Evaluating Chemicals for Endocrine Disruption, GD 150 (OECD, 2012): "If effects seen in existing lower level studies do not lead to adverse outcome in level 5 assay and if test is to current OECD 416 standards, no further testing needed. However, if test is not to current OECD 416 standards then consider supplemental testing, depending upon existing data".

Developmental effects such as increase in post-implantation loss and decrease in the number of live foetuses at 360 mg/kg/d dose occurred only in the presence of evident maternal toxicity (mortality and retarded body weight gain) in level 4 assay, Prenatal developmental toxicity study in rabbits (Anonymous, 1990) (for details please refer to RAR Volume 3, section B.6.6.2.2.). Additionally, it was concluded that this old study do not appear to comply with the updated OECD 414 (adopted 22nd January 2001) as main differences were identified: the dose period covered solely the period of major organogenesis (i.e. days 6-15 in the rat and days 7-19 in the rabbit), groups were with fewer than 16 animals with implantation sites at necropsy (rabbit study) and six- to ten-fold intervals of doses were used, instead of recommended two- to four-fold intervals..

A statistically significant increase in the incidence of thyroid follicular adenocarcinoma was observed in males at 20000 ppm (4/80; 5%) in level 4 assay, combined chronic toxicity and carcinogenicity study in. The increased incidence of the thyroid follicular adenocarcinoma at the top dose level was just at the upper edge of HCD range given and was above the average of HCD. However, the incidence of thyroid follicular adenocarcinoma in 2-year males only was slightly outside of historical range (4/70, 5.7%). The increased incidence of thyroid follicular adenocarcinoma was considered as incidental.

As part of the United States Environmental Protection Agency (US EPA) ToxCastTM program, trinexapac-ethyl was evaluated for potential effects in an extensive battery of *in vitro* assays aimed at identification of potential endocrine activity according to the notifier. The following types of studies/investigated endpoints were incorporated in the ToxCast battery regarding estrogenicity, androgenicity or thyroid effect: cell proliferation in T47D cells, protein-fragment complementation assays on estrogen receptor alpha (ERα) and beta (ERβ) homo- and heterodimerisation, ER/AR/TR transcription factor/mRNA transcription, AR mediated pathway activation, AR mediated pathway specific protein stabilization, Era/AR/TR-transactivation. In addition, enzyme (aromatase) inhibition assay was included in the ToxCast battery. Trinexapac-ethyl was negative in all of these assays.

According to the interim criteria in Annex II of Regulation (EC) No 1107/2009 for determining substances with endocrine disrupting properties, formally, trinexapac-ethyl is considered not to have endocrine disrupting properties on the basis that it is not or has not to be classified in accordance with the provisions of Regulation (EC) No 1272/2008 as carcinogen category 2 and toxic for reproduction category 2.

However, based on each of the relevant studies individually and a subsequent weight of evidence evaluation, it was concluded that an adverse effect of trinexapac-ethyl on the oestrus cycle via the hormonal system in 1-year dog study cannot be excluded what give rise to concern that trinexapac-ethyl might have endocrine disrupting potential. No mechanistic Level 2 data of the OECD framework were submitted for the evaluation of endocrine disruption properties of trinexapac-ethyl. In the absence of any other clear indications of endocrine-related adverse effects in the toxicological studies as well as in available ToxCast in vitro mechanistic data and in order to exclude any doubt on a possible endocrine activity of trinexapac-ethyl, this concern is considered to justify requests for further clarification of the ED potential using additional mechanistic data.

At the expert meeting (PPR 170, 11 – 14 December 2017), the majority of experts could not conclude on the ED properties based on available information of trinexapac-ethyl and suggested to provide in vitro assays (e.g. Steroidogenesis assay, OECD TG 456) and a comparative in vitro metabolism study between dog and human. The latter study would have been useful for further assessment of human relevance of dog findings and the potential role of metabolites.

2.6.9 Summary of medical data and information

The Occupational Health group of Syngenta has maintained a data base of incidents involving chemical exposure of workers since 1983 (for more detailed data please refer to RAR Volume 3, section B.6.9.). From 1994 data has been collected from all manufacturing, formulation and packing sites of Syngenta around the world. A query of the Syngenta internal database in June 2015 for trinexapac-ethyl resulted in zero records of adverse health reported from the handling of trinexapac-ethyl during synthesis and formulation activities. Control strategies are employed at all manufacturing facilities to reduce exposure and operator exposure limits are set. For trinexapac-ethyl, the current Syngenta Occupational Exposure Limit (OEL) value is 5 mg/m^3, equivalent to the agreed Syngenta maximum concentration for relatively non-toxic ‘nuisance dusts’. Trinexapac-ethyl has been handled in large quantities for over 20 years and with the use of appropriate control strategies, no adverse health effects associated with the material have been reported in the workforce.

Syngenta has kept detailed records of exposure and poisoning incidences on marketed products for many years from the USA, Canada and other cases. A review of the exposure incidences of trinexapac-ethyl formulations that have occurred between 2004 and 2012 has been conducted. 33 cases of occupational or accidental, 1 uncertain and 1 cases of intentional exposure related to trinexapac-ethyl have been recorded. Exposure happened through the dermal, oral, ocular, respiratory and unknown route. The majority of the reported cases were related to incidents with minor health symptoms (25 cases). Other cases have been reported with severity grade assignments of none (5), not followed (2) and moderate (3). The highest severity grade was moderate. All 3 cases were related to itching and burning symptoms after golfing at a golf course recently treated with trinexapac-ethyl containing products. The causal link of these incidents to trinexapac-ethyl exposure is unclear. The incident caused by intentional ingestion was leading to minor symptoms of temporary nature.

Trinexapac-ethyl is of low acute toxicity. Intoxication is only likely if large quantities are ingested. In animal studies, symptoms of acute poisoning were non-specific. From the reported incidences of human trinexapac-ethyl exposure the clinical symptoms observed were also transient and non-specific. Standard medical treatment is proposed with regard to eyes, skin, inhalation and ingestion.

2.6.10 Toxicological end points for risk assessment (reference values)

Table 68: Overview of relevant studies for derivation of reference values for risk assessment

Species	Study (method/type, length, route of exposure)	Test substance Batch No; purity	Critical effect	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Cross reference
Rat Tif: RAIf (SPF) hybrids of RII/1 x RII/2	**Short-term oral toxicity** 28-day oral, gavage partly in accordance with OECD 407 (1981)	Trinexapac-ethyl, LV 609024, 95%	↑ water consumption (M, F); ↑absolute and relative liver (M, F) & kidney (M) weight; liver and kidney histopathology (M)	100 (M)	1000/2000 (M)	Anonymous, 1988 Section 2.6.3
Rabbit, New Zealand White	**Short-term dermal toxicity** 22-day dermal, 6 h/d, semi-occlusive OECD 410 (1981)	Trinexapac-ethyl, FL 872026, 96.6%	No systemic effects	≥ 1000	Not obtained	Anonymous, 1989 Section 2.6.3
Rat, Sprague-Dawley	**Short-term oral toxicity** **90-day oral, dietary** **partly in accordance with OECD 408 (1981)**	**Trinexapac-ethyl,** **FL 872026, 96.6%**	**Histopathological kidney effects (M)**	**34 (M)**	**346 (M)**	Anonymous, 1989a Section 2.6.3
Beagle dog	**Short-term oral toxicity**	Trinexapac-ethyl, FL 872026, 96.6%	Clinical signs (emaciation) (M), ↓ body weight (M & F),	516	890	Anonymous, (1989b)

Species	Study (method/type, length, route of exposure)	Test substance Batch No; purity	Critical effect	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Cross reference
	90-day oral, dietary OECD 408 (1981)	FL 882373, 96.2% FL 881224, 94.6%	↓ body weight gain (M & F), ↓food consumption (M & F), ↓ absolute and relative thymus weight (M) & thymus atrophy (M & F) ↓bw (M: 26.1%; F: 11.7%) ↓bw gain: (M: -18.3%; F: -6.1%) ↓FC (M, F)			Section 2.6.3
Beagle dog	**Short-term oral toxicity** **1 year oral, dietary** **partly in accordance with OECD 452 (1981)**	**Trinexapac-ethyl,** **FL 882373, 96.2%** **FL 892178, 96.2%** **FL 891417, 92.2%**	**Clinical signs (faeces mucoid/bloody, M & F), ↓terminal bw (M: 11.5%), haematological changes (↓RBC, ↓HCT, ↓HGB) (F), possible effect on the oestrus cycle & decreased absolute uterus weight, brain histopathology (vacuolation) (M & F)**	**31.6**	**357.1**	Anonymous, 1992 four supplementary studies: B.6.3.2.3.1; B.6.3.2.3.2; B.6.3.2.3.3; B.6.3.2.3.4 Section 2.6.3
Rat, Sprague-Dawley	**Combined chronic toxicity /carcinogenicity study** 52/104-week oral, dietary OECD 453 (1981)	Trinexapac-ethyl, FL 872026, 96.9% FL 881224, 96.9% FL 882373, 96.2% FL 892178, 96.2% FL 891417, 92.2%	Long-term: Interim renal histopathological effects (hyaline droplets) and bile duct hyperplasia in the liver (M), galactoceles in mammary skin (F) Carcinogenicity: an increased incidence of rare tumours was considered as incidental	115.6 (Long-term) ≥ 805.7 (Carcinogenicity)	392.7 (Long-term)	Anonymous, 1992 Section 2.6.5
Mouse, Crl:CD-1(ICR)Br	**Carcinogenicity study** 78-week, dietary OECD 451 (1981)	Trinexapac-ethyl, FL 872026, 96.9% FL 881224, 96.9% FL 882373, 96.2%	Long-term: There were no adverse effects Carcinogenicity: There were no tumour incidences	≥ 911.8 (Long-term) ≥ 911.8 (Carcinogenicity)	Not obtained	Anonymous, 1991 Section 2.6.5
Rat, Sprague-Dawley	**Two-generation reproduction toxicity study** Oral: diet Approximate number of dose weeks: F0 – 22-25; F1 – 20-23 OECD 416 (1983)	Trinexapac-ethyl, FL 882373, 96.2% FL 892178, 96.2%	Parental: ↓bw gain premating (F0 males Day 0-91: 9.6%; F1 males Day 0-84: 10.5%; F0 female Day 0-91: 14.8%); ↓FC premating (F1 males: average 5.9%) Offspring: ↓bw (both sexes F1 pups: ~20%; F2 pups: ~24%); decreased survival index (F1 sexes combined: Day 4-21; F2 female pups: Days 0-4)	106.2 (Parental) 662.9 (Offspring)	662.9 (Parental) 1293.0 (Offspring)	Anonymous, 1991 Section 2.6.6.1
Rat, Sprague-	**Developmental toxicity (teratogenicity)**	Trinexapac-ethyl,	Did not cause adverse effects at highest dose tested	≥ 1000 (Maternal)	Maternal: Not obtained	Anonymous, 1988

Species	Study (method/type, length, route of exposure)	Test substance Batch No; purity	Critical effect	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Cross reference
Dawley	**study** Days 6-15 of gestation, gavage OECD 414 (1981)	P.705002, 96.6%		200 (Developmental)	Developmental: 1000 mg/kg bw/d:	Section 2.6.6.2
Rabbit, New Zealand White	**Developmental toxicity (teratogenicity) study** Days 7-19 of pregnancy, gavage OECD 414 (1981)	Trinexapac-ethyl, P.705002, 96.6%	Maternal: ↑mortality, retarded body weight gain to Day 15 Developmental: ↑ post-implantation loss; ↓ number of live foetuses	60 (Maternal & Developmental)	360 (Maternal & Developmental)	Anonymous, 1990 Section 2.6.6.2
Rat, Crl:CD(SD)	**Subchronic (13 week) dietary neurotoxicity study** 13 weeks oral, dietary OECD 424 (1997)	Trinexapac-ethyl, SMO8E551, 95.8%	No signs of neurotoxicity and systemic adverse effects observed at highest dose tested.	≥ 948 (Neurotoxicity & Systemic)	Not obtained	Anonymous, 2012a Section 2.6.7
Mouse (female), B6C3F1	**28-Day immunotoxicity feeding study** 28-days oral, dietary Immunotoxicity US EPA OPPTS 870.7800 (1998)	Trinexapac-ethyl, SMO5D180, 96.6%	. No signs of immunotoxicity (the humoral and innate immune response) and systemic adverse effects observed at highest dose tested.	≥ 1530.5 (Immunotoxicity & Systemic)	Not obtained	Anonymous, 2011 Section 2.6.8.2

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

For Annex I of Council Directive 91/414/EEC inclusion of trinexapac as laid down in the review report for the active substance trinexapac (SANCO/10011/06 final of 4 April 2006) and approved in the Commission Directive 2006/64/CE of 18 July 2006 an Acceptable Daily Intake (ADI) was established on the basis obtained from the 1 year dog study. The NOAEL in this study was 32 mg/kg bw/day, based on clinical signs, body weight, haematology and brain histopathology. An ADI value of 0.32 mg/kg bw/day was calculated taking into account a safety factor of 100.

No additional data have been provided (except three supplementary reports regarding dog studies) for re-evaluation of trinexapac-ethyl that would affect the basis of the derived reference value agreed upon for Annex I of Council Directive 91/414/EEC inclusion of trinexapac-ethyl as laid down in the review report for the active substance trinexapac (SANCO/10011/06 final of 4 April 2006) and approved in the Commission Directive 2006/64/CE of 18 July 2006. It should be mentioned that first approval conclusion and the LOAEL/NOAEL has been changed regarding two-generation reproduction toxicity study in rats due to reconversion from diet test substance concentration (ppm) to the achieved mean dose (mg/kg bw/day).

The lowest relevant NOAEL for deriving the ADI was 31.6 mg/kg bw/day from the one-year oral toxicity study in dogs. This NOEL for both sexes was based on adverse toxic effects the next higher dose group (357.1 mg/kg bw/day): clinical signs (mucoid/bloody faeces) in males and females, decreased terminal body weight in males, haematological findings (decreased RBC, haematocrit, haemoglobin) in females, changes in oestrus cyclicity, decreased absolute uterus weight as well as brain histopathology (cerebral vacuolation) in both sexes.

Based on the results obtained in the toxicological data included for this evaluation, the assessment factor of 100, which is generally applied in risk assessment of active substances in plant protection products, is considered sufficient to protect from adverse effects of the substance. An **ADI** of **0.32 mg/kg bw/day** (rounded value) can thus be derived from an NOAEL of 31.6 mg/kg bw/day and an assessment factor of 100 (31.6/100 = 0.316 or ~ 0.32). The ADI set during the previous review under Directive 91/414 thus remains.

At the expert meeting (PPR 170, 11 – 14 December 2017), the experts agreed to keep an ADI of 0.32 mg/kg bw per day based on the 1-year dog study.

2.6.10.2 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

For Annex I of Council Directive 91/414/EEC inclusion of trinexapac as laid down in the review report for the active substance trinexapac (SANCO/10011/06 final of 4 April 2006) and approved in the Commission Directive 2006/64/CE of 18 July 2006 an acute reference dose (ARfD) for trinexapac-ethyl was not allocated as it was not considered necessary due to the low acute toxicity of the substance. There were no indications of acute effects in repeated dose toxicity studies and any embryotoxic or developmental effects. It should be noted that there were the two treatment related mortalities (two females) at 360 mg/kg/d in the developmental toxicity study in rabbits and the first death occurred on day 13 (6 days after dosing). It is noteworthy that there were 4/6 and 1/6 mortalities in a preliminary study at 800 mg/kg bw/day and at 400 mg/kg bw/day, respectively. The mortalities were attributed to substance irritation of the stomach mucosa as the animals had haemorrhagic depressions in the stomach.

For this renewal assessment, a new acute and subchronic neurotoxicity studies as well as 28-day immunotoxicity toxicity study are also available. No acute effects were observed in these studies which can be likely considered to present an acute hazard at relevant doses.

After the reassessment of the original DAR, and based on all new available information, the RMS for the renewal of trinexapac-ethyl follows the previous opinion that **no ARfD is needed**, i.e. the conclusion from the previous review remains.

At the expert meeting (PPR 170, 11 – 14 December 2017), experts discussed if ARfD should be set using as starting point the NOAEL of 200 mg/kg bw per day in the rat developmental toxicity study, however the finding (i.e. increase in the litter incidence of asymmetrically shaped sternebrae) was observed at limit dose 1000 mg/kg bw per day. The effect might not have resulted from a single exposure, and the finding was of doubtful classification as malformation or variation.

The majority of experts expressed the opinion that setting of ARfD is not necessary.

2.6.10.3 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL (acceptable operator exposure level)

For Annex I of Council Directive 91/414/EEC inclusion of trinexapac as laid down in the review report for the active substance trinexapac (SANCO/10011/06 final of 4 April 2006) and approved in the Commission Directive 2006/64/CE of 18 July 2006 the Acceptable Operator Exposure (AOEL) was established on the basis obtained from the 90-day study in rat. The NOAEL in this study was 34 mg/kg bw/day, based on reduced food consumption and body weight gain; biochemical and histological kidney effects, increase in relative liver weight. The AOEL value of 0.34 mg/kg bw/day was calculated taking into account a safety factor of 100. This conclusion is also supported for the renewal of the trinexapac-ethyl (2016) despite the fact that in the current evaluation the NOAEL of 34 mg/kg bw/day for the 90-day study in rat is based on the histopathological effects on the kidney (tubular basophilia and tubular hyaline droplets) only.

Thought the lowest short-term NOAEL originated from a one year dog study, the exposure period in this study is clearly longer than the one expected for workers. The 90-day study in rat is considered to be more realistic starting point for toxicological worker risk assessment. On the other hand the NOAEL values from these two studies are very similar.

An **AOEL** of **0.34 mg/kg bw/day** can thus be derived from an NOAEL of 34 mg/kg bw/day and an assessment factor of 100 (34/100 = 0.34). The AOEL set during the previous review under Directive 91/414 thus remains.

At the expert meeting (PPR 170, 11 – 14 December 2017), the experts agreed to keep an AOEL of 0.34 mg/kg bw per day based on the 90-day rat study.

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

After the reassessment of the original DAR, and based on all new available information, the RMS considers that for the renewal of trinexapac-ethyl there is no need for setting an AAOEL. This conclusion is based on the same arguments as for the ARfD: no acute effects were observed in any of the studies which can be likely considered to present an acute hazard at relevant doses.

At the expert meeting (PPR 170, 11 – 14 December 2017), the majority of experts expressed the opinion that setting of ARfD is not necessary. The same conclusion is applicable for AAOEL.

2.6.11 Summary of product exposure and risk assessment

Trinexapac-ethyl 250 g/L ME (A8587F) is a micro-emulsion (ME) containing 250 g/L trinexapac-ethyl for use as a plant growth regulator in field crops. The toxicological studies (i.e., acute oral and dermal toxicity, skin and eye irritation studies) have been performed with the formulation A8587B. The acute inhalation and skin sensitisation studies have been conducted on A8587F. Information on the detailed composition of the precursor formulation A8587B and the representative formulation A8587F can be found in the volume 4. These formulations could be considered similar with regards to acute toxicity and irritation.

A8587F is of low toxicity in respect to acute oral, dermal and inhalation toxicity and is not irritating to the rabbit skin (based on weight of evidence analysis), nor is not a skin sensitiser. It was however irritating to the rabbit eye, and therefore a classification of Eye Irrit. 2, **H319** "Causes serious eye irritation" is proposed. The classification according to Regulation (EC) No 1272/2008 as amended is given in the table below. A classification of **STOT SE 3, H335** "May cause respiratory irritation" and the supplemental hazard information **EUH066** "Repeated exposure may cause skin dryness or cracking" is also recommended for the representative formulation A8587B.

Table 69: Summary of acute toxicity of A8587F

Parameter [Reference]	**Species**	**Result**	**Classification according to Regulation (EC) No 1272/2008 as amended**
Acute oral LD_{50} (Anonymous, 1991)	Rat	LD_{50} >3000 mg/kg	None
Acute dermal LD_{50} (Anonymous, 1991a)	Rat	LD_{50} >4000 mg/kg	None
Acute inhalation LC_{50} (Anonymous, 2016)	Rat	LC_{50} > 5.45 mg/L/4h (nose only, aerosol)	None
Acute skin irritation (Anonymous, 1991) *(supporting information)*	Rabbit	Non-irritant *(based on weight of evidence analysis)*	None
Acute eye irritation (Anonymous, 1991a)	Rabbit	Irritant	Eye Irrit. 2, H319
Skin sensitisation (Anonymous, 2009)	Guinea Pigs	Non-sensitising	None

No experimental data on dermal absorption of trinexapac-ethyl in A8587F have been generated therefore worst case, default dermal absorption values have been assumed in accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2012; 10(4):2665). Thus, using of the recommended default dermal absorption values of 25% for the concentrate and 75% for the in use dilution is considered appropriate in view of the concentrations of the active substance in the representative formulation and in the spray dilution according to the critical GAP use of Trinexapac-ethyl 250 g/L ME (A8587F).

Operator exposure arising from the use of A8587F is acceptable. Estimates based on surrogate data contained in the German Model (geometric mean) predict that the proposed use of A8587F through field crop sprayers will result in a level of systemic exposure to trinexapac-ethyl equivalent to 35.9% of the AOEL of 0.34 mg/kg bw/day for an operator without the need for PPE.

According to UK POEM operator exposure to trinexapac-ethyl is predicted to be 54.1% of the AOEL for operators wearing gloves during all operations.

According to Operator Outdoor Spray AOEM calculations, it can be concluded that the risk of exposure to trinexapac-ethyl for the operator using A8587F for the proposed uses is acceptable without the use of personal protective equipment (i.e. 41.6% of the AOEL of 0.34 mg/kg bw/day) but with the use of workwear which consist of coveralls or long-sleeved jackets and trousers that were made of cotton or cotton/polyester.

Additionally, on the basis of the classification of the product as an eye irritant (H319) and as EUH066 “Repeated exposure may cause skin dryness or cracking“ the use of a face shield and gloves for operator when handling the concentrate would be required.

The bystander and residential exposure estimations using the German guidance paper (2008) indicate that levels of exposure for bystander and resident will be within acceptable levels of the proposed systemic AOEL of trinexapac-ethyl. A first tier systemic exposure to bystanders results in 2.0% of the AOEL (adult) and 1.6% of the AOEL (child) applying the drift values for 1 m distance (2.77%). Systemic exposure to bystanders results in 0.21% of the AOEL (adult) and 0.17% of the AOEL (child)) applying the drift values for 10 m distance (0.29%). A first tier systemic exposure to resident results in 0.23% of the AOEL (adult) and 0.37% of the AOEL (child) applying the drift values for 1 m distance (2.77%). Systemic exposure to resident results in 0.1% of the AOEL (adult) and 0.17% of the AOEL (child) applying the drift values for 10 m distance (0.29%) (section B.6.4.2.).

Regarding resident child and adult exposure levels of 13.3% of the AOEL for the child and 4.9% of AOEL for the adult are derived using EFSA Guidance Exposure Calculator (version 30 Mar 2015). According to EFSA guidance (EFSA Journal 2014; 12(10):3874) no bystander risk assessment is required for PPPs with no potential acute systemic toxicity. Exposure in this case will be determined by average exposure over a longer duration, and higher exposures on one day will tend to be offset by lower exposures on other days. Therefore, exposure assessment for residents also covers bystander exposure (section B.6.4.2.).

The risk to workers undertaking crop inspection activities is considered acceptable. Estimates using German with the EUROPOEM II re-entry models and EFSA Guidance Exposure Calculator (version 30 Mar 2015) predict that the proposed use of A8587F will result in a level of systemic exposure to trinexapac-ethyl equivalent to 11% and 6.2% of the AOEL, respectively, for the unprotected worker wearing adequate work clothing (but no PPE) when re-entering treated areas to carry out crop inspection. It should be noted, that worker exposure is acceptable even without workwear based on EFSA Guidance Exposure Calculator (version 30 Mar 2015).

2.7 Residues

2.7.1 Summary of storage stability of residues

Studies investigating stability of residues during storage of samples in both plant and animal origin matrices were reviewed during trinexapac-ethyl Annex I inclusion process. A summary of all data is presented in Table 2.7.1-1.

Some cereal samples from the residue trials were stored up to 24.5 - 25.5 months. As the degradation of trinexapac is slow (in grain, 90% of trinexapac was recovered after 24 months), the applicant considers that there is no impact on the levels of trinexapac in the samples and the stability studies are sufficient to cover the proposed uses of this application. RMS agrees with EFSA that trials not adequately supported by storage stability (stored for 25.5 months) shall be excluded from the assessment, however samples stored for 24.5 months should be included in the assessment, as additional 2 weeks are not anticipated to have a significant impact on degradation. Residues of trinexapac (CGA 179500) in cereal grain as well as in oilseed rape seeds can be considered as stable for at least 24 months when stored at -18°C. Residues of trinexapac (CGA 179500) in wheat straw can be considered as stable for at least 12 months when stored at -18°C. It is also stable in animal tissues and milk for at least 3 and 4 months respectively under freezer storage at -18°C.

Table 2.7.1-1: Summary of storage stability of residues in plant and animal matrices

Commodity	**Storage stability - group**	**Storage stability**
Oilseed rape – rape seeds	High oil content	-18 ^{0}C for at least 24 months
Wheat grain	High starch content	-18 ^{0}C for at least 24 months
Wheat straw	No group	-18 ^{0}C for at least 24 12 months
Bovine muscle		-18 ^{0}C for at least 3 months
Bovine liver		-18 ^{0}C for at least 3 months
Bovine kidney		-18 ^{0}C for at least 3 months
Bovine milk		-18 ^{0}C for at least 4 months
Bovine fat (omental)		-18 ^{0}C for at least 3 months
Bovine blood		-18 ^{0}C for at least 3 months

Additionally, high temperature hydrolysis studies showed that metabolites CGA 313458, CGA 113745 and CGA 224439 were formed during processing. Therefore storage stability studies for these metabolites covering the length of storage in processing studies were submitted by TTF. Storage stability of metabolites CGA 313458, CGA 113745 and CGA 224439 was demonstrated for the following periods in the commodities listed in the Table 2.7.1-2 below when frozen (approximately -18°C).

Table 2.7.1 – 2: Summary of stability data for metabolites CGA313458, CGA 113745 and CGA 224439 in processed cereal commodities

Commodity	**Maximum Storage Period (month) for which stability was demonstrated**
New Data	

Commodity	Maximum Storage Period (month) for which stability was demonstrated		
	CGA313458	CGA 113745	CGA 224439
Wheat grain	12	Not stable after 30 days	12
Flour	3	Not stable after 30 days	12
Bran	6	Not stable after 30 days	12
Bread	6	Not stable after 30 days	12
Beer	12	Not stable after 30 days	12

Analytical method GRM020.14A for CGA113745 gave poor chromatography during the processing study so development work was carried out and the chromatography was improved. The improved chromatography was used in the storage stability study to analyse for CGA113745 in processed matrices and showed that CGA113475 was unstable in the presence of crop matrices - degrading to only 20% of the initial amount over 30 days. Thus it can be assumed that inaccurate levels of CGA113745 were found in both the pre-processed incurred grain samples and the processed commodities due to degradation in storage and poor chromatography including possible co-elution with other components. Any data regarding residue levels of CGA113745 in the processing studies on wheat and barley should be disregarded and have been struck through. Residue levels of this metabolite in RAC and processed commodities as well as processing factors should be further assessed.

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

<u>Metabolism in plants</u>

The plant metabolism of trinexapac-ethyl was carried out in four crops, representing two crop groupings – oilseeds (oilseed rape) and cereals (wheat, rice, grass). The application method was foliar for all these crops.

The representative use for trinexapac-ethyl in the EU is on barley and wheat.

All studies were performed using a cyclohexane ring radiolabelled form of trinexapac-ethyl ([^{14}C]-trinexapac-ethyl). No study was conducted using cyclopropane ring radiolabelled form of trinexapac-ethyl ([14C]-trinexapac-ethyl). In one trial on spring wheat (new data), the application rate was 1.69 times higher than the critical GAP proposed for wheat in Southern and Northern Europe (0.211 *vs* 0.125 kg a.s./ha) and 1.06 times higher than the critical GAP proposed for barley in Southern and Northern Europe (0.211 vs 0.200 kg a.s./ha). In remaining wheat and oilseed rape trials the application rate was in line with the critical GAP proposed for wheat and oilseed rape in Southern and Northern Europe.

Trinexapac-ethyl (CGA163935) is extensively degraded in wheat, oilseed rape, rice and grass by very similar biotransformation pathways. It should be noted, that original metabolism studies (from the DAR) on oilseed rape and wheat (Nicollier, 1991 and Krauss, 1993) are considered supplementary due to deviations from OECD 501. Trinexapac-ethyl was only detected at trace levels in wheat forage and in all parts of rice and to a higher extent in wheat roots. Metabolism proceeded via hydrolysis to the major metabolite trinexapac (CGA179500) up to 0.577 mg/kg 40 % TRR in wheat grain, followed by hydroxylation (forming hydroxylated CGA179500 (SYN548584**)**; 0.175 mg/kg representing 12.1 % TRR) and subsequent ring opening of the cyclohexane ring. Stepwise oxidation/decarboxylation yielded saturated and unsaturated tricarboxylated acids such as CGA275537 (tricarballylic acid; up to 0.91 mg/kg representing 17 % TRR in grass seeds), CGA312753 (aconitic acid; 0.058 mg/kg representing 35 % TRR in rice husks) and citric acid, all precursors to incorporation into the biosynthetic pool of natural products.

A secondary pathway proceeded via ring opening of the cyclohexane ring of parent leading to formation of CGA300405 (0.374 mg/kg representing 20.7 % TRR in wheat forage) and the mono ethyl esters of CGA275537 (tricarballylic acid; up to 0.206 representing 10.3 % TRR in wheat hay and 0.37 representing 17 % TRR in rice husks), CGA312753 (aconitic acid; up to 0.058 mg/kg representing 35 % TRR in rice husks). Further steps observed were aromatisation of the 6-membered ring of trinexapac and keto-enol tautomerism to 4-cyclopropanecarbonyl-3,5-dihydroxobenzoic acid CGA329773 (up to 0.03 representing 2.5 % TRR in rice grain and 11 % TRR in wheat grain – supplementary study) and NOA433257 (terephthalic acid; found only in grass up to 3.5 mg/kg representing 12 % TRR in seed screenings of grass) and reduction of CGA179500 to yield CGA351210 (found only in supplementary study of oilseed rape oil, pods and stalks up to 28 % TRR).

In the new metabolism studies provided for renewal, the following metabolites – trinexapac (CGA179500), CGA300405, tricarballylic acid (CGA275537) and hydroxylated CGA179500 (SYN548584) – were found in amounts more than 10 %TRR. In EU reviewed metabolism studies, the following metabolites – CGA329773, trans-aconitic acid CGA312753, SYN540405, CGA351210 and terephthalic acid NOA433257 – were found in amounts more than 10 %TRR.

Although not all metabolites were found in every plant species, all observed degradation and transformation steps (oxidation, decarboxylation, ring cleavage, conjugation) occurred in all crops. Therefore, the metabolic pathways are considered comparable in all crops.

Proposed metabolic pathway of trinexapac-ethyl in plants is presented in figure 2.7.2-1.

At the expert meeting (PPR 171, 13 – 15 December 2017) it was discussed whether the available information may be sufficient to conclude on metabolism in plants and animals, as all plant and animal metabolism studies were conducted exclusively with the benzene ring label and not with the cyclopropyl moiety label. A cleavage of parent compound was observed in the available metabolism studies. The fate of the split-off cyclopropyl moiety is unknown since not investigated. A hydrolysis study simulating processing confirms the cleavage of the parent molecule and shows formation of compound CGA224439. The axperts agreed that a data gap should be identified for primary crop metabolism data in cereals with cyclopropyl labelling to appropriately address the data requirements for at least the representative uses. Moreover, the potential for uptake of residues bearing the cyclopropyl moiety in rotational crops and their identity should be investigated.

A data gap was set - a plant metabolism study with the cyclopropyl label in the cereal/grass crop category.

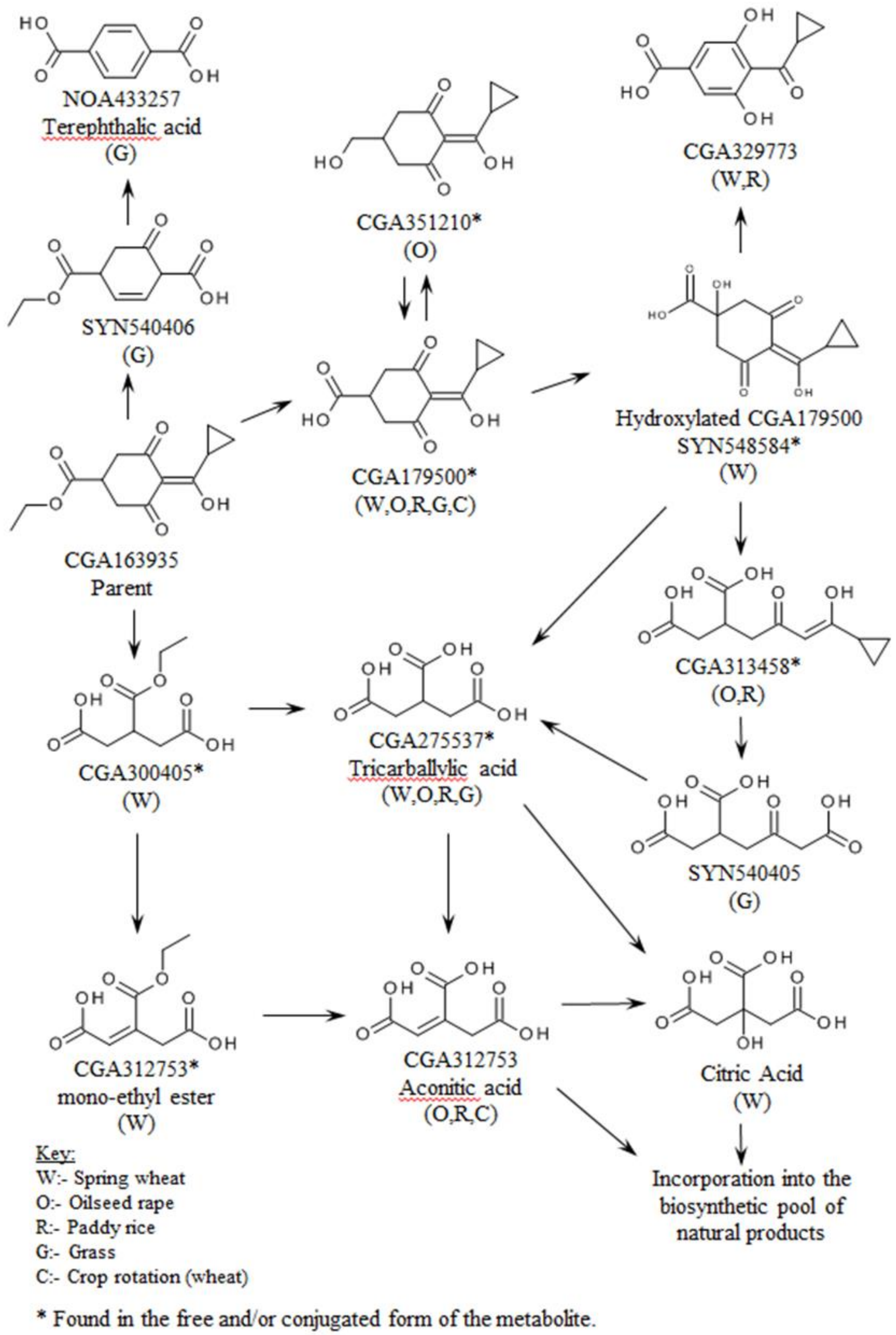


Figure 2.7.2-1: Proposed metabolic pathway of trinexapac-ethyl in plants

Metabolism in animals

The metabolism of CGA 163935 was studied in lactating goats and laying hens. In all metabolism studies ^{14}C-trinexapac-ethyl was used. However, it is noted that the metabolite CGA 179500, and also CGA 351210 (a further degradation product of CGA 179500, found only in supplementary metabolism study in oilseed rape), are the major residue components in livestock feed. As such, the livestock metabolism studies with trinexapac-ethyl might be considered less relevant in first instance. Considering the fast and extensive metabolism of trinexapac-ethyl to CGA 179500 as described below, the study results using trinexapac-ethyl are nevertheless taken into consideration.

A metabolism study in hen was reviewed for the inclusion in Annex I of Directive 91/414/EEC was considered supplementary during renewal. A new nature of residue study in hen was submitted to conform more realistic dose rates (0.85 mg/kg bw/d, still at 50 N rate for laying poultry) and longer period (4 compared to 10 days) than previous studies. The results from the new hen metabolism study demonstrated that, [^{14}C]-trinexapac-ethyl and/or its hens biotransformation products are readily excreted as more than 87% of the dose was accounted for in the excreta. Total radioactive residues in egg yolk and egg white reached a maximum level of 0.009 mg eq/kg and 0.031 mg eq/kg after 8 days of dosing, respectively. Egg white was the only sample found to contain residues >0.01 mg eq/kg. Parent trinexapac-ethyl and trinexapac were found in egg white at 0.005 mg/kg and 0.003 mg/kg respectively. Laying hens in the EU reviewed metabolism study, currently considered as supplementary, were dosed with 0.4 and 20.3 mg/kg bw/d. At the low dose, residues were below 0.01 mg eq/kg in eggs, and from 0.002 up to 0.043 mg eq/kg in tissues; at the high dosing level, residues were found in all tissues and eggs ranging from 0.095 to 1.77 mg eq/kg. The parent compound is found only in egg samples, especially in egg white, albeit the absolute levels are very low (up to 0.005 mg/kg in egg yolk and 0.12 mg/kg in egg white). Trinexapac (CGA 179500) is present in all tissue samples analysed, except egg white after high dosing. Trinexapac (CGA 179500) is accounting in most tissues for 60–84 % TRR (0.001 – 0.036 mg/kg) and 44-53% TRR (0.058 – 0.94 mg/kg) after high and low dosing, respectively. Results in supplementary and new (fully compliant with OECD 503) metabolism studies are similar. Two metabolism studies in lactating goat were reviewed for the inclusion in Annex I of Directive 91/414/EEC. During the re-evaluation for renewal one of the study (Müller 1993a) was considered supplementary. Following oral dosing for four consecutive days with trinexapac-ethyl at levels in the diet equivalent to 0.2, 3 and 20 mg/kg bw/d (17-1667 N rate) the majority of the administered dose was found in urine and faeces (66, 83 and 81% respectively for dose level). Only small amounts of the applied dose were found in milk (0.01, 0.05 and 0.02 % respectively for dose level) and edible tissues (3.27, 1.19 and 1.71% respectively for dose level) demonstrating that trinexapac-ethyl and its metabolites do not bio-accumulate and are rapidly excreted.

Parent trinexapac-ethyl was not detected. Trinexapac (CGA 179500) was the only major metabolite detected in all tissues and milk ranged from 0.004 to 34 mg/kg. CGA 113745 was the major metabolite detected in liver (0.13 mg/kg), in kidney (0.35 mg/kg) and in fat (0.012 mg/kg). This metabolite was found only in 3 mg/kg bw/d dose goat metabolism study and not found in other study (0.2 and 20 mg/kg bw/d, considered as supplementary) probably due to its long and not supported by storage data interval between sample and analysis.

Overall it is concluded that the metabolite CGA 179500 is the only residue component of significance in animal products. Excretion of the residue as CGA 179500 by both livestock species is fast and extensive. In addition, the livestock feeding studies performed with CGA 179500 indicate that at a nominal residue intake, no significant residue levels of CGA 179500 are expected. Based on these considerations, no additional livestock metabolism studies are necessary.

Since metabolism in rats and ruminants was demonstrated to be similar, the findings in ruminants can also be extrapolated to pigs.

At the expert meeting (PPR 171, 13 – 15 December 2017) it was agreed that in view of the importance of feed items from the intended uses and the expected residue levels, the nature of residues in livestock with regard to the cyclopropyl moiety should also be addressed.

A data gap was set - the nature of residues in livestock with regard to the cyclopropyl moiety should be addressed.

No metabolism study for fish was provided. The applicant's position is provided below *in italics*.

Document SANCO/10181/2013 Rev. 2.1, of 13 May 2013, states: In some cases, agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, waiving of these particular data requirement points is considered acceptable as long as no test methods or guidance documents are published in the form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02.

It is also recorded in the Summary Report of the Standing Committee meeting on Plants, Animal, Food and Feed (Section Phytopharmaceuticals - Pesticides Residues), held in Brussels on 24-25 November 2014, under item A.24, that " ... the Commission working document on the nature of residues in fish was discussed in 2013 and it was concluded that it is not yet finalised and ready to be noted as a guidance document." Additionally the report states under item A.24 the Commission emphasised that for the time being there are no agreed test guidelines and that hence the pertinent data requirements can be waived [as per document SANCO/10181/2013 Rev 2.1]."

In the Summary Report of the SCoPAFF meeting (Section Phytopharmaceuticals - Plant Protection Products - Legislation), held in Brussels on 26-27 January 2015, it is reiterated, under item A.26, "... some RMS are requesting studies on data requirements for which currently there is no agreed methodology and they consider a dossier incomplete if these data are not provided. The Commission explained that this is not consistent with the Guidance Document SANCO/10181/2013, which was taken note of by Member States." The following statements were also made by the Commission: "In particular cases, ad-hoc studies could be requested, as it is always the case in justified situations. ... However, the Commission referred to the general policy of reducing animal testing and asked Member States to consider this when asking for additional studies on vertebrates."

We believe that it is essential that guidance is suitably discussed and peer reviewed, considering both benefits to the assessment of consumer safety and the minimisation of vertebrate testing, before being applied.

In addition there are currently no definitive triggers in Regulation (EC) No. 283/2013 on which to base a decision as to whether a "fish metabolism" study is required or not.

***In order to properly assess the potential transfer of pesticide residues from plant-protection-product treated feed items into the consumable tissues of farmed fish we believe that the following need to be in place**:*

***A robust and representative dietary burden calculation method** (including the underlying feeding-practice data);*

***An agreed and practicable method for studying the nature of residues in fish**; and (depending on the potential for residues to transfer into fish tissues)*

***An agreed and practicable method for quantitatively studying the transfer of residues of concern into fish tissues**.*

RMS comments

The argument that no agreed test method or guidance is available is not considered a valid justification.

Detailed circumstances in which fish metabolism and feeding studies are triggered are described in SANCO/11187/2013. Although it is questionable if SANCO/11187/2013 can be used in this case, as this guidance shall be applied to all active substances that are fat soluble, i. e. substances with log Pow≥ 3, whereas trinexapac-ethyl is not fat soluble and log Pow is < 3.

According to Regulation 283/2013 “metabolism studies on fish may be required where plant protection product is used in crops whose parts or products, also after processing, are fed to fish and where residues in feed may occur from the intended applications”. As wheat (grain, bran, flour, germ, middlings and gluten) and barley (bran, brewer’s grain and distiller’s grain) are used for the formulation of aquaculture diets (SANCO/11187/2013), at least a dietary burden calculation should be provided showing if use of trinexapac-ethyl may lead to significant residues (generally considered to be > 0.1 mg/kg of the total diet (dry weight basis) in fish feed.

Proposed metabolic pathway of trinexapac-ethyl in livestock is presented in figure 2.7.2-2.

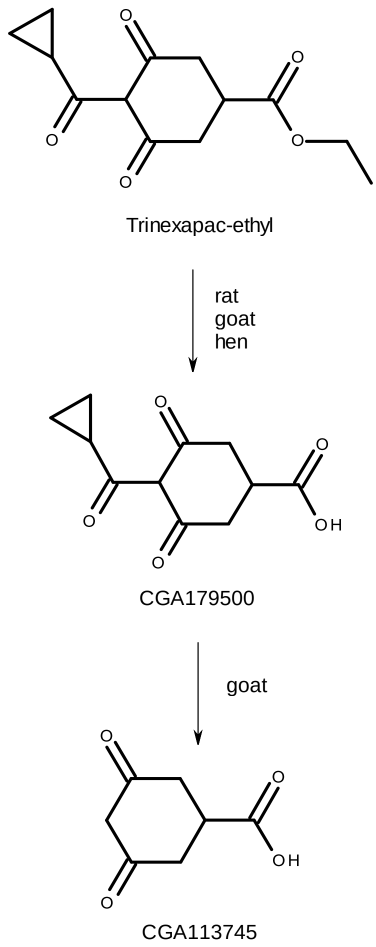


Figure 2.7.2-2: Proposed metabolic pathway of trinexapac-ethyl in livestock

2.7.3 Definition of the residue

In the process of Annex 1 listing under Directive 91/414/EEC (DAR, 2003), the residue definition for monitoring and risk assessment has been proposed as follows: Trinexapac and its salts in food of plant (cereals, only)". Only in the LoEP the simplified wording "Trinexapac (CGA 179500)" is used(EFSA, 2005), where trinexapac stands for trinexapac acid.

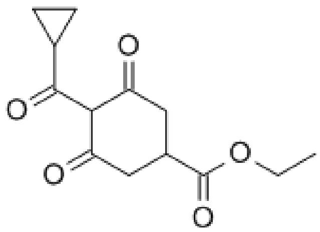
During the review of MRLs under Article 12 of Regulation (EU) No 396/2005, EFSA proposed the same residue definition "sum of trinexapac (acid) and its salts, expressed as trinexapac" where trinexapac stands for trinexapac acid (EFSA, 2012).

The analytical methods developed to measure trinexapac do not discriminate between residues of trinexapac undissociated acid from trinexapac salts (dissociated anions); the residues are determined as free trinexapac.

The additionally submitted metabolism studies (wheat and oilseed rape), a new rotational crop metabolism study and processing studies make a re-assessment of the residue definition necessary. A list of identified residues including their relative and absolute levels is given in the following tables. Results obtained from supplementary metabolism studies are underlined.

(1) Trinexapac-ethyl

Parent trinexapac-ethyl is relevant for inclusion into the residue definition for plants by default. In a new provided metabolism studies, trinexapac-ethyl was found only in 7 DAT forage of wheat and was not detected in any edible plant parts in any metabolism studies, exposure via feed can be excluded. It is proposed not to include trinexapac-ethyl in the definition of residue.

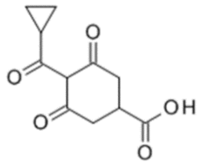
Codes and chemical names	Structure	Occurrence in metabolism (plant and animal) and rotational crop metabolism studies
CGA163935 Trinexapac-ethyl 4-(cyclopropanecarbonyl)-3,5-dioxo-cyclohexanecarboxylate		Dosed as trinexapac-ethyl: Wheat forage 7 DAT: 0.006 mg/kg, 0.3 % TRR Wheat roots: 0.26 mg/kg, 12.38 % TRR Rice foliage 7 DAT: 0.008 mg/kg, 5.5 % TRR Rice husks: 0.003 mg/kg, 1,8 % TRR Rice straw: 0.001 mg/kg, 0.9 % TRR Oilseed rape tops 30min: 1.2 mg/kg, 19 % TRR Oilseed rape green parts 14DAT: 0.012 mg/kg, 1.5 % TRR Oilseed rape flowering parts 14DAT: 0.068 mg/kg, 1 % TRR Egg white: 0.0017 mg/kg, 43 % TRR Egg white: 0.005 mg/kg, 31 % TRR Egg white: 0.12 mg/kg, 44 % TRR (high dose) Egg yolk: 0.005 mg/kg, 12 % TRR Rat faeces: 13, 22 and 39 % of TRR

(2) Trinexapac, free and conjugated (CGA179500)

Relevant for inclusion in residue definition, major metabolite in plant and animal matrices. In the new metabolism studies on wheat and oilseed rape, trinexapac (free and conjugated) is the main compound in oilseed rape, wheat forage and hay (~22% TRR) and wheat grain (40% TRR). Conjugates represented 2-3% TRR, except in wheat grain where they represented 12% TRR.

The toxicity of the trinexapac is considered covered by the studies conducted with the parent trinexapac-ethyl and no studies with this metabolite are considered necessary. It is subsequently followed that the trinexapac would not have any other toxicological properties than those observed in the toxicity studies with the active substance trinexapac-ethyl (Volume 1 Section 2.6.9.1).

Codes and chemical names	Structure	Occurrence in metabolism (plant and animal) and rotational crop metabolism studies

CGA179500 Trinexapac 4-(cyclopropanecarbonyl)-3,5-dioxo-cyclohexanecarboxylic acid		Found in all plant and animals metabolism studies up to 40 % TRR in plants and up to 96.8 % TRR in animals (refer to Volume 3 CA B.7 Table B.7.2.1-16 and Table B.7.2.2-1) Rat urine: 92 % TRR Rat faeces: 5, 50 and 79 % TRR

In processing studies residue levels of trinexapac (free and conjugated) ranged from 0.5–2.8 mg/kg in wheat grain and from 1.56–1.9 mg/kg in barley grain. Residue levels in processed commodities were all above the LOQ.

The median processing and conversion factors for processed commodities could not be derived for monitoring and risk assessment, as residue definition in processed commodities is open, pending the explanation the contradictory findings (stability vs. instability) in the standardised hydrolysis experiments

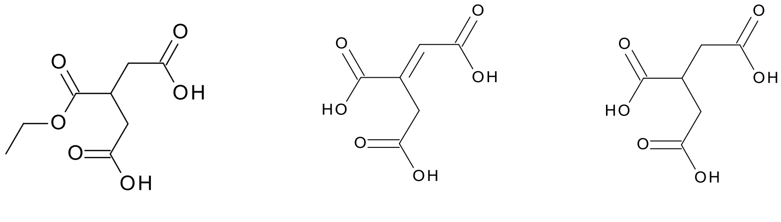
Residue levels of trinexapac (free and conjugated) have been measured in the submitted residue trials. Based on the results, it is proposed to consider trinexapac (free and conjugated) in the definition of residue for risk assessment. The proposed conversion factor is 2.6 for grain (*i.e.*, for the estimation of trinexapac (free and conjugated) from residue-level data for trinexapac (free form)).

(3) CGA300405

Minor metabolite, not relevant for inclusion into residue definition for plant or animal matrices except forage. This metabolite only occurs in wheat metabolism study, reaching 20.7 % TRR in forage 7 DAT and 0.8 % TRR in grain. Provisionally included in the definition of residue for risk assessment in cereal fodder items and grass (pending its toxicological relevance).

Based on the metabolism study, the conversion factors are 0.03 for grain and 2.73 for straw (*i.e.*, for the estimation from residue-level data for trinexapac (free form)). The residue levels in grain are anticipated to be below the LOQ (see Section B.7.3 of this document). The calculated highest residue level in straw is 0.87 mg/kg (barley, SEU).

This metabolite has not been found in livestock nor in the rat. However it is structurally similar to aconitic acid, which degrades into tricarballylic acid (CGA275537) in ruminants. It is anticipated that CGA300405 will undergo the same ester hydrolysis as aconitic acid (CGA312753) and will be degraded into tricarballylic acid.



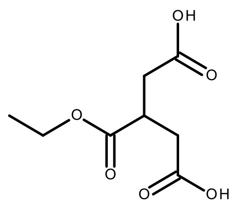
CGA300405 aconitic acid tricarballylic acid

The anticipated residue levels of CGA300405 in cereal straw are similar to those of tricarballylic acid. However, as the amount in forage is quite high (0.374 mg/kg, 20.7 % TRR), it is proposed to provisionally include this metabolite in the definition of residue for risk assessment in cereal fodder/grass items.

CGA300405 is considered to be non-mutagenic and non-clastogenic/aneugenic. Since there is no repeated toxicity study performed on CGA300405 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached. Due to the same reason the need of specific reference values in order to conduct a consumer risk assessment cannot be set (Volume 1 Section 2.6.9.1). At the mammalian toxicology expert meeting (PPR 170, 11 – 14 December 2017), it was concluded that the metabolite CGA300405 is not genotoxic.

At the residues expert meeting (PPR 171, 13 – 15 December 2017) the following data gap was set:

The relevance of metabolite CGA300405 in cereal crop feed items and the potential for residues in animal commodities should be further addressed.

Codes and chemical names	**Structure**	**Occurrence in metabolism (plant and animal) and rotational crop metabolism studies**
CGA300405 3-ethoxycarbonylpentanedioic acid		Dosed as trinexapac-ethyl: Wheat forage 7 DAT: 0.374 mg/kg, 20.7 % TRR Wheat hay: 0.161 mg/kg, 8.0 % TRR Wheat grain: 0.012 mg/kg, 0.8 % TRR Wheat straw: 0.131 mg/kg, 9.6 % TRR

(4) tricarballylic acid (CGA275537)

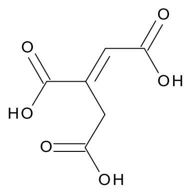
Tricarballylic acid (CGA275537) was observed in existing metabolism studies (up to 17% in grass seeds). It is also observed in the new metabolism study on wheat in significant amounts in wheat forage, hay and straw (7.8 to 10.3% TRR) and to a lesser extent in wheat grain (2% TRR, 0.03 mg/kg) and oilseed rape seeds (1% TRR, 0.004 mg/kg).

Based on the metabolism study, the conversion factors are 0.07 for grain and 2.31 for straw (i.e., for the estimation from residue-level data for trinexapac (free form)). The residue levels in grain are anticipated to be below the LOQ (see Volume 3 CA B.7.3). The calculated highest residue level in straw is 0.74 mg/kg (barley, SEU).

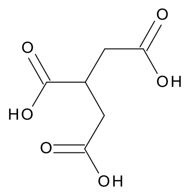
Tricarballylic acid is a natural product from the plant carbon pool, related to the citric acid cycle. Intake of tricarballylic acid from treated commodities is restricted to cereals straw. Comparing intakes based on residues in wheat straw (0.11 mg/kg, after a 1.7N treatment, equivalent to 0.06 mg/kg at 1N dose), the intakes of tricarballylic acid from the use of trinexapac-ethyl will be a fraction of the one naturally occurring in grass and therefore no adverse effects in ruminants should be expected.

Regarding exposure of tricarballylic acid, the applicant refers to assessment report of prohexadione calcium, which is another plant growth regulator approved in Europe (France, 2009). This assessment is based on bibliography - Nelson and Mottern (1931), Meirion (1951) and Russel (1989). However, these studies were not provided for re-assessment to the RMS LT by the applicant. During the peer review of prohexadione-calcium, the exposure of tricarballylic acid was assessed, conclusions can be summarised the following way:

- tricarballylic acid is a ruminant metabolite formed from trans aconitic acid (also named CGA312753);



aconitic acid



tricarballylic acid

- observed levels of trans aconitic acid in crops range between 2 and 6%;
- levels of trans aconitic acid above 1% in grass leads to toxicity;
- trans aconitic acid is approximately converted to 40% into tricarballylic acid by ruminants. This gives a theoretical “toxic” residues in grass of >4000 mg/kg tricarballylic acid.

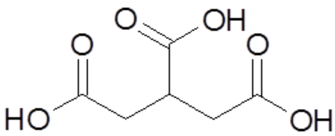
Intake of tricarballylic acid from treated commodities is restricted to cereals straw. Comparing intakes based on residues in wheat straw (0.11 mg/kg, after a 1.7N treatment, equivalent to 0.06 mg/kg at 1N dose), the intakes of tricarballylic acid from the use of trinexapac-ethyl will be a fraction of the one naturally occurring in grass and therefore no adverse effects in ruminants should be expected.

Table 2.7.3-2: Estimated livestock dietary intake of tricarballylic acid

Commodity	Residues of tricarballylic acid (mg/kg)	Dry matter (%)	Residue level on dry weight (mg/kg)	Contribution of feed item to the livestock diet (% of total diet mass, DM basis)	Residue contribution (mg/kg bw/d)
Grass (naturally occurring level at which adverse effects have been observed)	4000	25	16000	Beef cattle: 50 Dairy cattle: 60 Ram/ewe: 95 Lamb: 50	Beef cattle: 192 Dairy cattle: 369 Ram/ewe: 507 Lamb: 340
Straw (from trinexapac-ethyl)	0.74	89	0.83	Beef cattle: 30 Dairy cattle: 30 Ram/ewe: 60 Lamb: 60	Beef cattle: <0.01 Dairy cattle: <0.01 Ram/ewe: 0.02 Lamb: 0.02

Since consumers are already exposed to this compound through natural sources, no further consideration of its toxicity is required and it cannot be considered appropriate for monitoring purposes since it could be detected as a natural product and not from the use of Trinexapac-ethyl, even though the available data on general toxicity (acute oral toxicity) demonstrated that this metabolite is of higher toxicity than the parent substance . There were no structural alerts noted following Ames test. Since there is no repeated toxicity study performed on CGA275537 (Tricarballylic acid) a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached. Due to the same reason the need of specific reference values in order to conduct a consumer risk assessment cannot be set (see Volume 1 Section 2.6.9.1). At the mammalian toxicology expert meeting (PPR 170, 11 – 14 December 2017), it was noted that to rule out the genotoxic potential of the metabolite CGA275537 more than one QSAR prediction tool together with read-across should be applied as predictions by a single model is not sufficient. Experts concluded that further data will be needed to conclude on the genotoxic potential of the metabolite (chromosomal aberration endpoint) and repeated exposure if risk assessment is triggered by the residues experts. Metabolite GA300405 (3-ethoxycarbonylpentanedioic acid, see below) seems to be an ester of tricarballylic acid and read-across for genotoxicity between the two might be applied (data gap: further analysis of the read-across should be performed).

It is proposed not to include tricarballylic acid in the definition of residue.

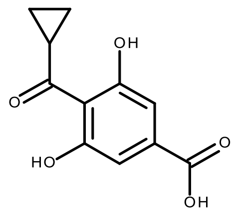
Codes and chemical names	**Structure**	**Occurrence in metabolism (plant and animal) and rotational crop metabolism studies**
CGA275537 Tricarballylic acid 1,2,3-Propanetricarboxylic acid		Dosed as trinexapac-ethyl: Oilseed rape seeds: 0.004 mg/kg, 1.0 % TRR Wheat forage 7 DAT: 0.141 mg/kg, 7.8 % TRR Wheat hay: 0.206 mg/kg, 10.3 % TRR Wheat grain: 0.03 mg/kg, 2.0 % TRR Wheat grain: 0.014 mg/kg, 3.1 % TRR Wheat straw: 0.111 mg/kg, 8.1 % TRR Wheat straw: 0.01 mg/kg, 2.4 % TRR Rice foliage 7 DAT: 0.006 mg/kg, 4.0 % TRR Rice foliage 21 DAT: 0.003 mg/kg, 3.9 % TRR Rice grain: 0.04 mg/kg, 3.2 % TRR Rice husks: 0.005/0.37 mg/kg, 3.2/17 % TRR Rice straw: 0.031/0.21 mg/kg, 19/13 % TRR Grass forage 22 DAT: 0.28 mg/kg, 14.0 % TRR Grass forage 102 DAT: 0.005 mg/kg, 9.3 % TRR Grass straw: 0.81 mg/kg, 16.8 % TRR Grass seeds: 0.91 mg/kg, 17.0 % TRR Grass seed screenings: 1.2 mg/kg, 16.0 % TRR

(5) CGA329773

This metabolite was not detected in newly provided metabolism studies on wheat (grain) and oilseed rape. It was detected exceeding 10 % TRR only in “old” metabolism study with wheat (0.05 mg/kg or 11 % TRR in grain), which was conducted with some deviations from the guidelines and considered only as supplementary. In was observed in other wheat and rice matrices at amounts not exceeding the 8.1 % TRR.

The available data on general toxicity –short-term toxicity study in rats - clearly demonstrated that the compound might be considered less toxic than the parent substance (Volume 1 Section 2.6.9.1).

Therefore metabolite is considered as minor and not relevant for inclusion in the residue definition for plants or animals.

Codes and chemical names	**Structure**	**Occurrence in metabolism (plant and animal) and rotational crop metabolism studies**
CGA329773 4-(cyclopropanecarbonyl)-3,5-dihydroxy-benzoic acid		Dosed as trinexapac-ethyl: Wheat forage 7 DAT: 0.012 mg/kg, 0.7 % TRR Wheat hay: 0.027 mg/kg, 1.4 % TRR Wheat grain: 0.05 mg/kg, 11 % TRR Wheat straw: 0.002 mg/kg, 0.1 % TRR Wheat straw: 0.016 mg/kg, 3.1 % TRR Rice grain: 0.003/0.03 mg/kg, 2.9/2.5% TRR Rice husks: 0.001/0.03 mg/kg, 0.7/1.2 % TRR Rice straw: 0.01 mg/kg, 0.8 % TRR

(6) hydroxylated CGA179500 (SYN548584)

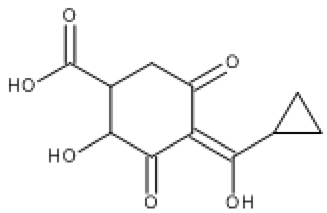
In the new metabolism studies on wheat, a compound has been characterised as a hydroxylated form of trinexapac. However the position of hydroxylation has not yet been established with certainty during submission of the dossier. OH-trinexapac has been recovered in all wheat matrices but is predominantly found in grain (12% TRR, 0.175 mg/kg). This metabolite was not included in the reference compounds in any other metabolism study.

As analytical standards are not yet available, OH-CGA179500 could not been measured in the residue trials.

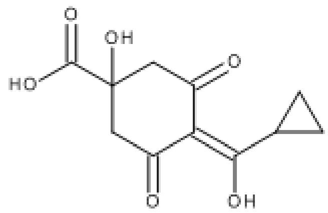
Statements from a position paper due to OH group position in this metabolite provided by the applicant on 31 January 2017, is stated below *in italics*:

The position of the OH group in the hydroxylated trinexapac acid component could not be confirmed by chromatographic means as no reference standard was available, however LC-MS/MS analysis and chemical characterisation has enabled the applicant to conclude on the structure of this metabolite. Following conduct of the GLP study, non-GLP work was initiated to attempt to determine the position of hydroxylation. This was carried out by isolation of the component of interest from the grain commodity to produce a sample of sufficient purity for analysis by NMR. This has been unsuccessful due to large amounts of endogenous material co-eluting with the component of interest.

In parallel to this work, attempts to synthesis the two proposed hydroxylated trinexapac acid (1-hydroxy-trinexapac acid and 2-hydroxy-trinexapac acid) components have been ongoing.



2-hydroxy



1-hydroxy

To date, the diastereoisomer pairs of the 2-hydroxy metabolite have been synthesised (SYN549426 and SYN549427). Analysis by two dissimilar chromatographic systems (HPLC and 2D-TLC) both indicate that they do not match the component of interest in grain. Attempts to synthesise the tertiary alcohol have to date been unsuccessful. Based on the data provided above and confirmation that the 2-hydroxy component is not present, the grain metabolite is identified as the 1-hydroxy metabolite (SYN548584).

Although OH-CGA179500 was not observed in the rat, no alerts were identified for genotoxicity (using (Q)SAR analysis (DEREK, LHASA Ltd)). A final conclusion on the genotoxic potential cannot be drawn for this metabolite based on the information provided (please refer to Volume 1 Section 2.6.9.1 and Volume 3 CA B.6 for further details).

Due to toxicological profile of hydroxylated trinexapac, the applicant provided a position, that:

"In terms of general toxicity, hydroxylated trinexapac acid is structurally similar to trinexapac acid (CGA179500), and trinexapac-ethyl.

Hydroxy trinexapac acid differs from CGA179500 in the addition of a hydroxyl group on the cyclohexane ring. The addition of a hydroxyl group is unlikely to result in increased toxicity, and may make the metabolite more readily excreted (the hydroxyl group may be available for conjugation and aid rapid excretion). CGA179500 is the major rat metabolite of trinexapac-ethyl, and therefore the toxicity profile of trinexapac-ethyl effectively covers both molecules. Both hydroxy trinexapac acid potential metabolites would be expected to be of equivalent or possibly lower toxicity than trinexapac-ethyl/CGA179500.

Trinexapac-ethyl is non-genotoxic, of low acute oral toxicity, and neither carcinogenic or reproductively toxic. Therefore the hydroxylated trinexapac acid metabolites would be expected to be of similarly low concern.

Despite exhaustive attempts to isolate and/or synthesise the hydroxylated CGA179500 – identified by default as SYN548584- it has not been possible. The molecule appears to be unstable outside the plant matrix and reverts to CGA179500.

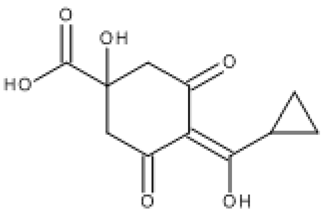
As the toxicity of this molecule is likely to be equivalent to trinexapac-ethyl and of low concern and cannot be synthesised, Syngenta propose that it is removed from the definition of the residue for risk assessment in plant matrices."

Consequently, a GLP study (Piskorski R. 2017) with the aim to confirm whether an unidentified metabolite in a wheat grain commodity (reported as "Hydroxylated CGA179500") from an IES Study # 20120098: Metabolism of [14C]-Trinexapac-ethyl in Spring Wheat co-chromatographs with supplied reference standards was provided by the applicant and included in Vol. 3 B.7.2.1 as study 8. RMS LT agrees with the conclusion that the reference standards used in this study (two diastereoisomers of the 2-hydroxy-metabolite) and these structures have been ruled out by co-chromatography, and therefore, the radioactive residues identified as the hydroxylated CGA179500 metabolite can be assigned to the 1-hydroxy-CGA179500 named as SYN548584

The identity of this compound was not fully confirmed (by exclusion of any other possible structure). In view of this uncertainty and the requirement of a new metabolism study with the cyclopropyl label, further elucidation/confirmation of the identity and amounts of this compound is awaited, before a final decision can be taken regarding its relevance as a residue in cereal grains (expert meeting, PPR 171, 13 – 15 December 2017).

In terms of general toxicity, hydroxylated trinexapac is structurally similar to trinexapac (CGA179500), and trinexapac-ethyl. Hydroxy trinexapac differs from CGA179500 in the addition of a hydroxyl group on the cyclohexane ring. The addition of a hydroxyl group is unlikely to result in increased toxicity, and may make the metabolite more readily excreted (the hydroxyl group may be available for conjugation and aid rapid excretion). Based on the available information, it can be assume that the toxicity of the hydroxylated CGA179500 is covered by the trinexapac (CGA179500) as well as the parent trinexapac-ethyl (CGA163935). Please refer to Volume 1 Section 2.6.9.1.

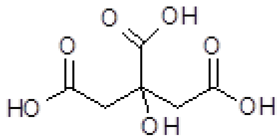
Taking into account that SYN548584 is unstable, could not be synthesised, analytical method is not available and its toxicity is covered by parent and trinexapac, it is proposed not to include metabolite SYN548584 in the residue definition.

Codes and chemical names	**Structure**	**Occurrence in metabolism (plant and animal) and rotational crop metabolism studies**
Hydroxylated CGA179500 (SYN548584) Hydroxylated trinexapac 4-[cyclopropyl(hydroxy)methylene]-1-hydroxy-3,5-dioxo-cyclohexanecarboxylic acid		Dosed as trinexapac-ethyl: Wheat forage 7 DAT: 0.06 mg/kg, 3.3 % TRR Wheat hay: 0.102 mg/kg, 5.1 % TRR Wheat grain: 0.175 mg/kg, 12.1 % TRR Wheat straw: 0.026 mg/kg, 1.9 % TRR

(7) citric acid

Minor plant metabolite found in wheat straw only (new metabolism study). Metabolite was not included in the reference compounds in any other metabolism study. Since wheat straw is an inedible commodity and it was not observed in animal metabolism study, the metabolite is not considered relevant for an inclusion into the residue definition for plants or animals.

The RMS considers citric acid a toxicologically non-relevant metabolite based on rationale given in Volume 3 B-6 (Volume 1 Section 2.6.9.1, for details please refer to point B.6.8.1.11).

Codes and chemical names	**Structure**	**Occurrence in metabolism (plant and animal) and rotational crop metabolism studies**
Citric acid		Dosed as trinexapac-ethyl: Wheat straw: 0.027 mg/kg, 2.0 % TRR

(8) Cyclopropane carboxylic acid (CPCA) CGA224439

This metabolite was only found in high temperature hydrolysis studies, representing up to 17.7 % TRR. Therefore new processing studies on wheat and barley were conducted in 2015 in order to measure CGA224439 magnitude in processed commodities. The studies were conducted at an elevated rate (2N for barley, 3.2N for wheat).

CPCA was recovered in low amounts in the grain (0.02-0.05 mg/kg) and in the processed commodities in the following low amounts:

- <0.01-0.03 mg/kg in all barley processed products, except bran (0.12 mg/kg) and brewers' yeast (0.11 mg/kg);
- <0.01-0.05 mg/kg in all wheat processed products, except dry gluten (0.08 mg/kg).

However, these residue levels are not significant when compared to the initial residue levels of trinexapac; the processing factors derived are consequently all very low. These preliminary processing factors derived range from 0.01 to 0.06, which demonstrate that residues of CPCA are not likely to be present in the processed commodities (Table 2.7.3-3).

The definitive processing factors and conversion factors could not be derived for processed commodities, as the definition for residue in processed commodities is still open (pending the explanation the contradictory findings (stability vs. instability) in the standardised hydrolysis experiments).

Table 2.7.3-3: Preliminary processing factors for cyclopropane carboxylic acid

Processed Commodity	**Median PF***
Barley, pot	0.01
Barley, pearled	0.01
Barley, bran	0.06
Barley, flour	0.01
Barley, brewing malt	0.01
Barley, malt sprouts	0.02
Barley, brewers' grain	0.01
Barley, brewers' yeast	0.05

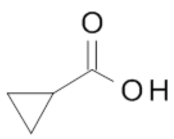
Barley, beer	0.01
Wheat, waste (offal)	0.02
Wheat, bran	0.02
Wheat, shorts	0.01
Wheat, middlings	0.01
Wheat, white flour	0.01
Wheat, wholemeal flour	0.02
Wheat, wholemeal bread	0.02
Wheat, germ	0.02
Wheat, dry gluten	0.03
Wheat, dry starch	0.01
Wheat, gluten feed meal	0.01

*Processing Factor calculated as residue of CPCA in processed product/residue of total trinexapac in RAC

Nonetheless, a conservative exposure assessment (TTC approach, which is not considered acceptable) has been conducted and provided by the applicant with these processing factors. However, input values (STMR for wheat and barley grain) used in these calculations are different from the ones calculated by RMS. Residue definition for processed commodities is still open, therefore chronic and acute exposure for CPCA was not recalculated by RMS and was removed from Vol 1.

CPCA is considered to be non-genotoxic. According to the additional literature search cyclopropane carboxylic acid has the pyruvate metabolism disruption properties, is considered a hypoglycemic agent and therefore it could potentially make CPCA more toxic than parent. Though a 90-day rat study on CPCA (Carpenter C., 2012) is referred to in the JMPR review of active substance aminocyclopyrachlor (JMPR, 2014), it has not been submitted to the RMS for an independent assessment. The data on short-term toxicity study in rats clearly demonstrate that the metabolite CPCA might be considered of higher toxicity than the parent substance trinexapac-ethyl. A conclusion on the general toxicity cannot be drawn for this metabolite as no data was provided. Since there is no repeated toxicity study performed on CGA224439 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached. Due to the same reason the need of specific reference values in order to conduct a consumer risk assessment cannot also be set (see Volume 1 Section 2.6.9.1 and Volume 3 CA B.6 for further details).

As the consumer risk assessment could not be finalised, residue definition in processed commodities is open, pending the explanation the contradictory findings (stability vs. instability) in the standardised hydrolysis experiments, the possible inclusion of metabolite CPCA in the definition of residue could not be concluded.

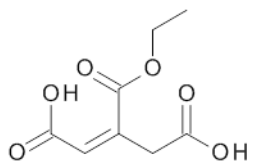
Codes and chemical names	**Structure**	**Occurrence in metabolism (plant and animal) and rotational crop metabolism studies**
CGA224439 Cyclopropane carboxylic acid		Dosed as trinexapac-ethyl: High temperature hydrolysis: Found in all conditions at 5.4 – 17.7 % TRR Magnitude of residues in processed

		commodities (max values): Barley, grain: 0.04 mg/kg Barley, pot barley: 0.02 mg/kg Barley, pearled barley: 0.02 mg/kg Barley, bran: 0.12 mg/kg Barley, flour: 0.03 mg/kg Barley, brewing malt: 0.01 mg/kg Barley, malt sprouts: 0.03 mg/kg Barley, brewers grain: 0.01 mg/kg Barley, brewers yeast: 0.11 mg/kg Barley, beer: 0.02 mg/kg Wheat, all matrices: 0.01 – 0.05 mg/kg Wheat, dry gluten: 0.08 mg/kg

(9) Aconitic acid CGA312753

This metabolite was not detected in the new provided wheat and oilseed rape metabolism studies. This is a minor metabolite in old metabolism studies, found in small both percentage and actual amounts, and reaching 35% TRR only in rice husks (not used for food or feed). Metabolite was also found in rotational crop metabolism study with wheat at very low actual amount (0.001 - 0.002 mg/kg). A conclusion on the general toxicity cannot be drawn for this metabolite as no data was provided.

Therefore metabolite is not considered relevant for an inclusion into the residue definition for plants or animals.

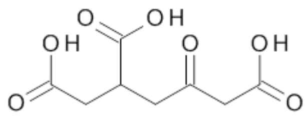
Codes and chemical names	Structure	Occurrence in metabolism (plant and animal) and rotational crop metabolism studies
CGA312753 mono-ethyl ester (Z)-3-ethoxycarbonylpent-2-enedioic acid		Dosed as trinexapac-ethyl: <u>Wheat husks: 0.02 mg/kg, 4.3 % TRR</u> <u>Wheat straw: 0.01 mg/kg, 1.8 % TRR</u> Wheat forage (rotational): 0.001 mg/kg, 10.0 % TRR Wheat hay (rotational): 0.002 mg/kg, 18.2 % TRR Rice foliage: 7 DAT: 0.004 mg/kg, 2.5 % TRR Rice foliage: 21 DAT: 0.002 mg/kg, 2.6 % TRR Rice grain: 0.007 mg/kg, 8.0 % TRR Rice husks: 0.058/0.02 mg/kg, 35/1.1 % TRR <u>Oilseed rape seeds: 0.013 mg/kg, 0.9 % TRR</u> <u>Oilseed rape seeds meal: 0.013 mg/kg, 0.9 % TRR</u> <u>Oilseed rape pods: 0.06 mg/kg, 0.9 % TRR</u> <u>Oilseed rape stalks: 0.047 mg/kg, 1.5 % TRR</u>

(10) Metabolite A - SYN540405

Minor plant metabolite found in grass only (old metabolism study). Metabolite was not included in the reference compounds in any other metabolism study. Grass is not a representative use.

At the expert meeting (PPR 171, 13 – 15 December 2017) the following data gap was set:

Further information should be submitted regarding the relevance of unique metabolites A, B and C identified in the grass study at significant levels, with a view to comprehensively address the metabolism for the entire category of cereal/grass crops.

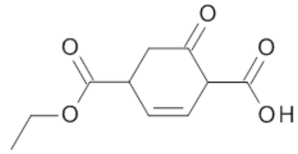
Codes and chemical names	Structure	Occurrence in metabolism (plant and animal) and rotational crop metabolism studies
SYN540405 4-oxopentane-1,2,5-tricarboxylic acid		Dosed as trinexapac-ethyl: Grass forage 22 DAT: 0.15 mg/kg, 7.4 % TRR Grass forage 102 DAT: 0.002 mg/kg, 4.4 % TRR Grass straw: 0.48 mg/kg, 10 % TRR Grass seeds: 0.1 mg/kg, 1.9 % TRR Grass seed screenings: 0.27 mg/kg, 3.8 % TRR

(11) Metabolite B - SYN540406

Minor plant metabolite found in small amounts in grass only (old study). Metabolite was not included in the reference compounds in any other metabolism study. Grass is not a representative use.

At the expert meeting (PPR 171, 13 – 15 December 2017) the following data gap was set:

Further information should be submitted regarding the relevance of unique metabolites A, B and C identified in the grass study at significant levels, with a view to comprehensively address the metabolism for the entire category of cereal/grass crops.

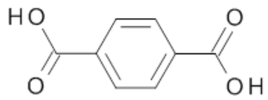
Codes and chemical names	Structure	Occurrence in metabolism (plant and animal) and rotational crop metabolism studies
SYN540406 4-ethoxycarbonyl-6-oxo-cyclohex-2-ene-1-carboxylic acid		Dosed as trinexapac-ethyl: Grass forage 22 DAT: 0.17 mg/kg, 8.6 % TRR Grass forage 102 DAT: 0.001 mg/kg, 2.7 % TRR Grass straw: 0.27 mg/kg, 5.6 % TRR Grass seeds: 0.46 mg/kg, 8.3 % TRR Grass seed screenings: 0.70 mg/kg, 9.9 % TRR

(12) Metabolite C - NOA433257

Minor plant metabolite found in grass only (old study). Metabolite was not included in the reference compounds in any other metabolism study. Grass is not a representative use.

At the expert meeting (PPR 171, 13 – 15 December 2017) the following data gap was set:

Further information should be submitted regarding the relevance of unique metabolites A, B and C identified in the grass study at significant levels, with a view to comprehensively address the metabolism for the entire category of cereal/grass crops.

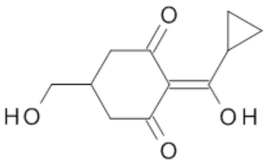
Codes and chemical names	**Structure**	**Occurrence in metabolism (plant and animal) and rotational crop metabolism studies**
NOA433257 Terephthalic acid		Dosed as trinexapac-ethyl: Grass forage 22 DAT: 0.20 mg/kg, 9.8 % TRR Grass forage 102 DAT: 0.004 mg/kg, 6.6 % TRR Grass straw: 0.45 mg/kg, 9.4 % TRR Grass seeds: 0.53 mg/kg, 9.6 % TRR Grass seed screenings: 3.5 mg/kg, 12 % TRR

(13) CGA351210

Metabolite was only found in supplementary metabolism study on oilseed rape. This metabolite represented 16 % TRR in oilseed rape oil, but with small relative amount (0.005 mg/kg). In rape matrices not used for food or feed, this metabolite represented up to 28 % TRR (taking into account free and conjugated forms). Oilseed rape is not a representative use.

A conclusion on the genotoxic potential and/or on the general toxicity cannot be drawn for this metabolite as no data was provided.

Metabolite is not considered relevant for an inclusion into the residue definition for plants or animals.

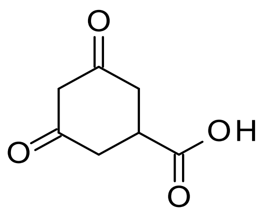
Codes and chemical names	**Structure**	**Occurrence in metabolism (plant and animal) and rotational crop metabolism studies**
CGA351210 2-[cyclopropyl(hydroxy)methylene]-5-(hydroxymethyl)cyclohexane-1,3-dione		Dosed as trinexapac-ethyl: Oilseed rape seeds: 0.077 mg/kg, 5.5 % TRR Oilseed rape oil: 0.005 mg/kg, 16.0 % TRR Oilseed rape seeds meal: 0.073 mg/kg, 5.2 % TRR Oilseed rape pods: 1.07 mg/kg, 16.0 % TRR Oilseed rape stalks: 0.87 mg/kg, 28.0 % TRR

(14) CGA113745

Metabolite found in goat metabolism study at levels up to 16.3 % TRR. It was also found in high temperature hydrolysis studies, representing up to 11.6 % TRR. Therefore new processing studies on wheat and barley were conducted in 2015 in order to measure CGA113745 magnitude in processed commodities. The studies were conducted at an elevated rate (2N for barley, 3.2N for wheat). The residue levels of CGA113745 in beer were below the LOQ. Nevertheless all residue results are not covered by storage stability data and the metabolite is proven to be unstable. Although CGA113745 was found to be unstable in brewing and baking samples (wheat grain, flour, bran, beer and bread) stored under frozen storage conditions. Only 20% CGA113745 was found after 30 days whereas samples were analysed after maximum of 15 months of storage. Analytical method GRM020.14A for CGA113745 gave poor chromatography during the processing study so development work was carried out and the chromatography was improved. The improved chromatography was used in the storage stability study to analyse for CGA113745 in processed matrices and showed that CGA113475 was unstable in the presence of crop matrices - degrading to only 20% of the initial amount over 30 days. Thus it can be assumed that inaccurate levels of CGA113745 were found in both the pre-processed incurred grain samples and the processed commodities due to degradation in storage and poor chromatography including possible co-elution with other components. Therefore any data regarding residue levels of CGA113745 in the processing studies on wheat and barley should be disregarded. Residue levels of this metabolite in RAC and processed commodities as well as processing factors should be further assessed.

Metabolite CGA113745 is unlikely to be genotoxic, however having higher eye damage and skin irritation/sensitisation potency than the parent substance. The available data on the short-term toxicity study in rats demonstrated that the metabolite CGA113745 is of comparable / equal short-term toxicity than the parent substance. Therefore, the reference values of the parent can be applied to CGA113745 (see Volume 1 Section 2.6.9.1 and Volume 3 CA B.6 for further details).

The possible inclusion of metabolite CGA 113745 in residue definition in processed commodities should be further assessed when data on magnitude in RAC and processed commodities will be available. As the metabolite was present in goat tissues at significant amounts, it was included in the definition of residues for risk assessment for ruminant commodities.

Codes and chemical names	**Structure**	**Occurrence in metabolism (plant and animal) and rotational crop metabolism studies**
CGA113745 REF 347-01 Cyclodione acid 3,5-dioxocyclohexanecarboxylic acid		Dosed as trinexapac-ethyl: Goat liver: 0.13 mg/kg, 16.3 % TRR Goat kidney: 0.35 mg/kg, 6.0 % TRR Goat fat: 0.012 mg/kg, 11.4 % TRR High temperature hydrolysis: Found in all conditions at 9.6 – 11.6 % TRR

(15) CGA313458

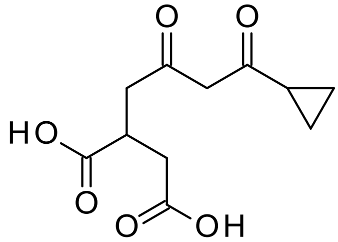
Minor metabolite found in rice and supplementary oilseed rape metabolism studies. In the new provided wheat and oilseed rape metabolism studies this metabolite was not detected.

This metabolite was also found in high temperature hydrolysis studies, representing up to 21 % TRR. Therefore new processing studies on wheat and barley were conducted in 2015 in order to measure CGA 313458 magnitude in processed commodities. The studies were conducted at an elevated rate (2N for barley, 3.2N for wheat).

Both processing studies showed that residue levels of CGA313458 were below the LOQ (0.01 mg/kg) in virtually all matrices studied (except in one beer sample where it was found at 0.01 mg/kg and wholemeal bread where it was found at 0.01-0.02 mg/kg). Therefore no processing factor has been derived for this metabolite as is it not present in significant quantity in any of the commodities studied. Although it should be noted, that the metabolite CGA 313458 was shown to be stable for only 3 months on flour, 12 months in grain and 6 months in bran and bread, any data regarding residue levels of this metabolite in flour, bran and bread in the processing studies on wheat and barley should be disregarded and have been struck through. Residue levels of CGA 313458 in flour, bran and bread as well as transfer factor in to flour, bran and bread should be assessed further.

Metabolite is considered not genotoxic and available data on general toxicity (acute toxicity) demonstrated that this metabolite was of similar acute toxicity than the parent substance. At the mammalian toxicology expert meeting (PPR 170, 11 – 14 December 2017), it was concluded that the metabolite CGA313458 is not genotoxic. Since there is no repeated toxicity study performed on CGA313458 a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached. Due to the same reason the need of specific reference values in order to conduct a consumer risk assessment cannot also be set (see Volume 1 Section 2.6.9.1 and Volume 3 CA B.6 for further details).

It can therefore be concluded that there is an insignificant exposure potential for this metabolite and considering the available toxicological data, it can be excluded from the residue definition for RAC plant and animals. In terms of processed commodities, the conclusion of possible inclusion in the definition of residue could not be finalised, as residue definition in processed commodities is open, pending the explanation the contradictory findings (stability vs. instability) in the standardised hydrolysis experiments.

Codes and chemical names	**Structure**	**Occurrence in metabolism (plant and animal) and rotational crop metabolism studies**
CGA313458 REF 361-01 R3A 2-(4-cyclopropyl-2,4-dioxo-butyl) butanedioic acid		Dosed as trinexapac-ethyl: Rice foliage 1 h: 0.012 mg/kg, 2.2 % TRR Rice foliage 7 DAT: 0.007 mg/kg, 5.1 % TRR Rice foliage 21 DAT: 0.002 mg/kg, 2.6 % TRR Rice grain: 0.04 mg/kg, 3.3 % TRR Rice husks: 0.16 mg/kg, 7.4 % TRR Rice straw: 0.007/0.12 mg/kg, 4.6/7.2 % TRR Oilseed rape seeds: 0.015 mg/kg, 1.1 % TRR Oilseed rape seeds meal: 0.015 mg/kg, 1.1 % TRR Oilseed rape pods: 0.127 mg/kg, 1.9 % TRR

		Oilseed rape stalks: 0.152 mg/kg, 4.9 % TRR High temperature hydrolysis: Found in all conditions at 3.8 - 21 % TRR Magnitude of residues in processed commodities: Barley, beer: 0.01 mg/kg Barley, all other matrices: <0.01 mg/kg Wheat, all other matrices: <0.01 mg/kg

(16) Evaluation of processing metabolites CGA 113745 and CGA 313458

For improved clarity, evaluation of processing metabolites taken from the original DAR 2005 with added comments of RMS LT is provided below.

The information (*in italics*) is taken from a statement submitted by the notifier (Twomey and Greener, 2004: trinexapac ethyl: toxicological relevance of metabolites CGA 113745 and CGA 313458). This information *in italics* was not re-evaluated by the mammalian toxicology section during renewal procedure and provided here only for transparency.

"To determine the possible effect of processing on CGA 179500, it was subjected to hydrolysis conditions representative of those typical of common industrial processes. Under all sets of conditions measured, CGA 179500 undergoes degradation, but it remains the major component at the end of the experiment with > 50% of total radioactive residue (% TRR). The other metabolites identified at levels greater than 10% TRR were CGA 313458 (15.8-20.6% TRR) and CGA 113745 (9.6-11.4% TRR).

CGA 313458 has been identified as a plant metabolite. CGA 113745 has not previously been identified in plants, rats or hens but has been identified in a recent goat metabolism study.

CGA 313458

CGA 313458 has been identified as a metabolite of parent compound CGA 163935 in both rice and rape at levels of <5% TRR in the edible portions of these plants. It is a precursor of aconitic acid (CGA 312753), which is an element of the citric acid cycle (Krebs cycle) and is integrated by de novo synthesis into the plant matrix.

The acute LD_{50} for CGA 313458 is greater than 2000 mg/kg and it is negative in the Ames test.

DEREK (structure based) analysis of CGA 313458 gave four alerts: mutagenicity (CGA 313458 is negative in the Ames test) and skin sensitisation were the same as for the parent compound (CGA 169395), which were falsely predicted as shown by in vivo data, plus alerts for carcinogenicity and gastric irritation. However, as CGA 313458 is a precursor to elements of the citric acid cycle (aconitic acid), this would imply that humans and animals are naturally exposed to this and similar structures within the plant matrix in the diet without adverse effects. In addition, aconitic acid is contained in the FDA List of Food Additives that is "Generally Regarded As Safe". As a naturally occurring endogenous compound, aconitic acid is considered to be of no toxicological concern. Therefore it is considered that CGA 313458, in the small quantities that would be produced by hydrolysis would also be of no toxicological significance.

CGA 113745

CGA 113745 has not previously been identified in plants, rats or hens but was identified at low levels in the liver, kidney and fatty tissue in a recent goat metabolism study. It was also detected at low levels in the hydrolysis study.

DEREK (structure based) analysis of CGA 113745 gave two alerts: mutagenicity and skin sensitisation that were the same as for the parent compound (CGA 163935) and the primary metabolite CGA 179500. These endpoints were falsely predicted in vivo for these compounds and were therefore considered tested in vivo for CGA 113745.

Structurally, CGA 113745 is very similar to a manufacturing intermediate (CGA 158377), for which there is a toxicology package up to a 28-day repeat dose toxicity study in the rat. The No Effect Level (NOEL) for this study was 100 mg/kg/day and the No Adverse Effect Level (NOAEL) for this study was 1000 mg/kg/day. The Ames and IVC studies for CGA 158377 were negative. As it is plausible that CGA 158377 would be converted to CGA 113745 in vivo, the NOEL for the 28 day study and the in vitro study results for CGA 158377 are considered to reflect those of CGA 113745.

For animals that were exposed to CGA 158377, the most likely route of metabolism would be rapid O de-ethylation to CGA 113745 (this is the primary metabolic step for the parent CGA 163935 to CGA 179500). CGA 113745 is a highly polar, water-soluble molecule, which would be rapidly excreted via the urine. It is therefore considered that animals administered CGA 158377 have already been systemically exposed to CGA 113745 and that a NOEL of 100 mg/kg/day is applicable for this compound."

Evaluation by RMS Netherlands, 2005.

Assessments of the potential exposure to both CGA 313458 and CGA 113745 through the diet have been carried out by the notifier. The calculation of the amount of both CGA 313458 and CGA 113745 potentially present in processed commodities were made from the residue data taking into account the results of the hydrolysis study and the processing factor obtained from processing studies. As wheat grain processed into flour/bread, and barley grain processed into beer are composite samples, STMR values were used in assessing the possible residues of CGA 179500 in the grain prior to processing. The maximum expected concentration of CGA 113745 in processed fractions was based on STMRs of CGA 179500, processing factors for CGA 179500 mentioned in the List of Endpoints, and the mean % TRR at which the metabolites CGA 313458 and CGA 113745 were found in the high temperature hydrolysis studies. In addition, potential residues of CGA 113745 in animal products were estimated based on the maximum residue of CGA 113745 in offal (liver) and the proposed MRL for non-poultry offal (0.05 mg/kg instead of 0.02 mg/kg; worst case). In the calculations, a worst-case situation was assumed where all wheat grain is eaten as bread and all barley grain is consumed as beer.

These estimates resulted in the following intakes (TMDI, mg/kg bw/day):

CGA 313458, Dutch diet: 0.000018 (general population), 0.000043 (children).

CGA 313458, WHO/GEMS diet: 0.000027.

CGA 113745, Dutch diet: 0.000011 (general population), 0.000025 (children).

CGA 113745, WHO/GEMS diet: 0.000017.

Toxicological relevance of CGA 313458 (RMS Netherlands)

The fact that this metabolite is a precursor of aconitic acid (an endogenous plant compound) is considered to be insufficient ground to conclude that CGA 313458 itself is of no toxicological significance, as this does not imply that humans will be naturally exposed to CGA 313458. Considering however the very low intake (at the most 0.000043 mg/kg bw/day for children (Dutch diet)), further information on general toxicity of the metabolite is not required. However, the non-genotoxic potential of CGA 313458 should be demonstrated in *in vitro* mammalian cell mutagenicity tests (mammalian cell chromosome aberration test and mammalian cell gene mutation test).

Comments RMS LT:

For revewal, *in vitro* mammalian cell mutagenicity tests were provided.

CGA313458 was found to be of low acute oral toxicity LD50 >2000 mg/kg bw for rats. Consequently, the available data on general toxicity (acute toxicity) demonstrated that this metabolite was of similar acute toxicity than the parent substance.

No evidence of genotoxicity was seen in an Ames test. A negative response was also observed In vitro Mammalian Cell Gene Mutation study with Chinese hamster V79 cells (HPRT) and in vitro chromosome aberration assay with human lymphocytes. It is therefore considered that CGA313458 is not genotoxic. The metabolite CGA313458 is a precursor of aconitic acid (an endogenous plant compound), i.e. element of the citric acid cycle, however, this does not imply that humans will be naturally exposed to this and similar structures within the plant matrix in the diet (see Volume I Section 2.6.9.1 and Volume 3 CA B.6 for further details).

New processing studies on wheat and barley were conducted in 2015 in order to measure CGA 313458 magnitude in processed commodities (see B.7.5.3 of this document). The studies were conducted at an elevated rate (2N for barley, 3.2N for wheat).

Both processing studies showed that residue levels of CGA313458 were below the LOQ (0.01 mg/kg) in virtually all matrices studied (except in one beer sample where it was found at 0.01 mg/kg and wholemeal bread where it was found at 0.01-0.02 mg/kg). Therefore no processing factor has been derived for this metabolite as is it not present in significant quantity in any of the commodities studied. Although it should be noted, that samples of flour, bread and bran matrices in these studies are not covered by storage stability data. Residue levels of CGA 313458 in flour, bran and bread as well as transfer factor in to flour, bran and bread should be assessed further.

Toxicological relevance of CGA 113745 (RMS Netherlands)

It is accepted that toxicology data for CGA 158377 will reflect those of CGA 113745, as the primary metabolic step in the metabolism of CGA 158377 is likely to be hydrolysis of the ethyl ester bond yielding CGA 113745. The complete toxicology package for CGA 158377 however (including the full set of mutagenicity studies: Ames test, mammalian cell chromosome aberration test and mammalian cell gene mutation test) has not been submitted (data requirement).

Comments RMS LT:

For renewal, the above mentioned data requirement were fulfilled.

Combined with the Ames and in vitro chromosome aberration data on CGA158377 as well as mammalian gene mutation data on CGA113745 it can be concluded that both CGA113745 and CGA158377 are unlikely to be genotoxic.

The available data on general toxicity of CGA158377 demonstrated that the metabolites (CGA113745 and CGA158377) are of comparable acute/short-term toxicity however they have higher eye damage and skin irritation/sensitisation potency than the parent substance. The available data on the short-term toxicity study in rats demonstrated that the metabolite CGA113745 and CGA158377 are of comparable / equal short-term toxicity than the parent substance. Therefore, the reference values of the parent can be applies to CGA113745 and CGA158377 (see Volume 1 Section 2.6.9.1 and Volume 3 CA B.6 for further details).

New processing studies on wheat and barley were conducted in 2015 in order to measure CGA 113745 magnitude in processed commodities (see Volume 3 CA B.7.5.3). The studies were conducted at an elevated rate (2N for barley, 3.2N for wheat).

Both processing studies showed that residue levels of CGA113745 were below the LOQ (0.01 mg/kg) in virtually all matrices studied (except in barley bran samples where it was found at 0.01 mg/kg). Nevertheless all residue results in these studies are not covered by storage stability data and the metabolite is proven to be unstable in the presence of crop matrices - degrading to only 20% of the initial amount over 30 days. Thus it can be assumed that inaccurate levels of CGA113745 were found in both the pre-processed incurred grain samples and the processed commodities due to degradation in storage and poor chromatography including possible co-elution with other components. Therefore any data regarding residue levels of CGA113745 in the processing studies on wheat and barley should be disregarded. Residue levels of this metabolite in RAC and processed commodities as well as processing factors should be further assessed.

Definition of the residue in plants

Based on the results from the metabolism studies, at the expert meeting (PPR 171, 13 – 15 December 2017) it was agreed that in view of the pending data request for a new metabolism study in cereals with the cyclopropyl label and further clarification on metabolites, the RD for RA for primary crops - the cereal/grass crop category should be provisional and should include:

- for cereal grains trinexapac, free and conjugated.

• for cereal fodder items /grass trinexapac, free and conjugated plus CGA300405 (expressed as trinexapac or separate, pending its toxicological relevance)

For monitoring of residues in the cereal/grass crop category: trinexapac and its salts, expressed as trinexapac.

A data gap was set - a plant metabolism study with the cyclopropyl label in the cereal/grass crop category.

Based on the metabolism study, the conversion factor is 2.2 for cereal grain (i.e., for the estimation from residue-level data for trinexapac (free form)).

Definition of the residue in livestock

At the expert meeting (PPR 171, 13 – 15 December 2017) it was agreed that awaiting further information on the nature of residues in livestock with regard to the cyclopropyl moiety, the RD for RA should be set as follows:

• Poultry : trinexapac;

• Ruminant: trinexapac plus metabolite CGA 113745, expressed as trinexapac;

Monitoring: trinexapac and its salts, expressed as trinexapac;

A data gap was set - the nature of residues in livestock with regard to the cyclopropyl moiety should be addressed.

Definition of the residue in processed commodities

It was originally proposed by the applicant to include processing metabolites in residue definition for risk assessment for processed commodities (processing commodities: trinexapac (free and conjugates), CGA313458, CGA113745 and CPCA (tentative)). After completion of magnitude processing studies, it was decided to remove these processing metabolites from the residue definition (processing commodities: trinexapac (free and conjugates). Nevertheless a separate exposure assessment of this processing metabolite (CPCA) was provided by the applicant but not accepted by the RMS (Volume 1 Section 2.7.9). All residue results for metabolite CGA 113745 are not covered by storage stability data and the metabolite is proven to be unstable. Residue levels of this metabolite in RAC and processed commodities as well as processing factors should be further assessed

RMS was of the opinion that the same residue definition for raw and processed commodities should apply, as proposed residue definition for raw agricultural commodities covers the one proposed for processed commodities. Although in the storage stability study appeared that the levels of metabolite CGA 113745 in RAC and processed commodities are not clear, CF could not be derived and possible inclusion of this metabolite in definition for RA in processed commodities could not be decided and will be discussed at the expert meeting. For metabolite CPCA a conclusion if the metabolite is of lower, equal or higher toxicity than the parent cannot be reached. Due to the same reason the need of specific reference values in order to conduct a consumer risk assessment cannot also be set. For risk assessment - tentatively proposed by RMS as sum of trinexapac and its salts (free and conjugates), with possible inclusion of CGA 113745 (unclear amount in RAC and processed commodities due to instability), CGA313458 (unclear amount in bran, flour and bread due to instability) and

CPCA (impossible risk assessment due to unclear reference values). For monitoring –sum of trinexapac and its salts, expressed as trinexapac is proposed.

From the expert meeting (PPR 171, 13 – 15 December 2017) :

To address the effect of food processing conditions on residues, four standard hydrolysis studies were submitted showing partially contradictory outcomes. Two studies were suggesting the stability of trinexapac-ethyl and trinexapac, respectively under hydrolysis conditions while the other two studies showed significant degradation under baking and sterilisation conditions. The experts were unable to conclude on the relevant residues in processed commodities. Further clarification by the applicant to explain the ambiguous findings in this standardised experiment is necessary. Also a data gap was set - further clarification should be submitted by the applicant to explain the contradictory findings (stability vs. instability) in the standardised hydrolysis experiments.

Residue definition for processed commodities remained open pending the explanation on the contradictory findings (stability vs. instability) in the standardised hydrolysis experiments.

2.7.4 Summary of residue trials in plants and identification of critical GAP

Representative use

The representative uses supported in the framework of Directive 91/414/EEC was a single post-emergence (foliar) treatment in cereals at a rate of 0.2 kg as/ha at BBCH 49 and BBCH 30-39 in northern and southern EU respectively.

Critical GAP

Referring to section 1.5.1 the critical GAP for the use of trinexapac-ethyl is based on an application rate of 0.2 kg as/ha at the developing stage of BBCH 25-49 of winter barley, on an application rate of 0.15 kg at the developing stage of BBCH 25-37 in spring barley, and on an application rate of 0.125 kg/ha at the developing stage of BBCH 25-49 in winter wheat seed rape in southern EU as presented in the table 2.7.4-1.

Table 2.7.4-1: Critical GAPs in EU supported by the applicants in the renewal application

Crop	Region	Pests	Outdoor/Indoor	Method	Timing of application	No. of applications	Rate per treatment kg as/ha	PHI
Winter Barley	EU	Prevention of lodging	F	Foliar spray	BBCH 25-49	1	0.2	-
Spring Barley	EU	Prevention of lodging	F	Foliar spray	BBCH 25-37	1	0.15	-
Winter wheat	EU	Prevention of lodging	F	Foliar spray	BBCH 25-49	1	0.125	-

The representative crops in the original EU review of trinexapac-ethyl also included cereals. New trials and data are presented for these crops to replace the data originally evaluated. The new residue trials were conducted in order to measure trinexapac, both free and conjugated forms since conjugates were observed in significant levels in the plant metabolism study (see Volume 3 Section B.7.2.1). Residue trials evaluated under Directive 91/414/EEC are not relied on in the framework of this submission because:

- they only measured the free form of trinexapac;
- some trials were not conducted at the proposed GAP;
- some trials were considered deficient due to the lack of raw data in the reports.

Studies in barley

Fifteen trials have been conducted in northern (8 at-harvest trials in Germany, France, UK and Belgium) and southern (7 at-harvest trials in Italy, Spain and France) Europe on barley at the critical GAP have been performed during the seasons of 2012 - 2015. In order to provide a complete dataset for southern Europe, the residue levels from the processing study (two trials) conducted at 1×400 g a.s./ha (i.e. 2X) were adjusted to take account of the application (proportionality principle), it should be noted that all NEU and SEU datasets were scaled to 1 N rate (GAP rate), details of those studies are presented in Volume 3 section B.7.5.3. Total of eight trials in NEU and nine trials in SEU were provided by the TTF. Two trials from NEU were not covered by storage stability and therefore excluded from the assessment, also two trials from UK (YO176QA and YO627TD) were considered as replicated as conducted only 10 km apart, leading to total of 5 trials in NEU. Two trials from SEU (IT, 27010 and 26866) were considered as replicates as conducted only 15 km apart, leading to and 8 trials in SEU acceptable for the assessment.

As the use pattern is intended for grain production only, residue data on forage are not required. It should be noted that the definition for risk assessment for cereal grain and cereal fodder are different and proposed as provisional.The residues of trinexapac (free) in barley grain in NEU ranged from <0.01 mg/kg to 0.12 mg/kg, in SEU ranged from <0.01 mg/kg to 0.49 mg/kg.

Studies in wheat

Twenty trials have been conducted in northern (12 at-harvest trials in Germany, France, UK, Austria, Czech Republic and Poland) and southern (8 at-harvest trials in Italy, Spain and France) Europe on wheat at the critical GAP (1x125 g a.s./ha, with the application being made at BBCH 49). Twelve trials have been conducted in northern Europe because the eight residue trials conducted in 2015 were located around two main geographical points (although these latter were more than 30 km apart). Moreover, the residue levels from the processing study (two trials in southern Europe) conducted at 1×400 g a.s./ha (i.e. 3.2X) were scaled down taking account of the proportionality principle to provide a larger and statistically more robust dataset, details of those studies are presented in Volume 3 section B.7.5.3. It should be noted that all NEU SEU datasets were scaled to 1 N rate (GAP rate). Total of twelve trials in NEU and ten trials in SEU were provided by the applicant. Two trials from NEU (FR, 60490 and 60113) were considered as replicates as conducted only 9 km apart, leading to total of 11 trials in NEU and 10 trials in SEU acceptable for the assessment.

As the use pattern is intended for grain production only, residue data on forage are not required. It should be noted that the definition for risk assessment for cereal grain and cereal fodder are different and proposed as provisional.The residues of trinexapac (free) in wheat grain in NEU ranged from 0.03 mg/kg to 0.39 mg/kg, in SEU ranged from 0.03 mg/kg to 0.27 mg/kg.

MRL application

Rye

The notifier has requested a modification of the existing EU MRLs on rye. No trials were provided by the applicant.

The data to support an increase of MRL for this crop have been submitted to support the representative uses on barley and wheat. Indeed, according to the guidance document SANCO 7525/VI/95 rev 10.2, data on wheat can be extrapolated to oat, rye and barley.

The representative use pattern for evaluation of the use on rye is detailed in Table 2.7.4-2.

Table 2.7.4-2: Critical GAPs for trinexapac-ethyl use on rye

Crop	**Residue region**	**Outdoor/ Protected**	**Growth Stage**	**Maximum Number of Applications**	**Minimum Application Interval (days)**	**Maximum**		**Minimum PHI (days)**
						Rate (L product/ha) [kg a.s./ha]	**Water (L/ha)**	
Rye	NEU	Outdoor	BBCH 25-49	1	n.r.	0.5 [0.125 kg a.s./ha]	100-400	n.r.
Rye	SEU	Outdoor	BBCH 25-49	1	n.r.	0.5 [0.125 kg a.s./ha]	100-400	n.r.

The critical GAP is the same than the critical GAP on wheat (see Table 2.7.1-2), therefore wheat data are considered acceptable to derive MRLs and risk assessment values. All data are provided in Volume 3 CA B.7.3.2.

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

Dietary burden

The representative use of trinexapac-ethyl is barley and wheat where both might be fed to livestock. The median and maximum dietary burdens for livestock were calculated using the OECD methodology (OECD, 2013). The input values for the dietary burden calculation were selected according to the latest FAO recommendations (FAO, 2009) and are summarised in Table 2.7.5-1. As the residue definition in processed commodities is open, for wheat milled by-products, wheat gluten and brewers/distillers grain, the default processing factors have been included in the calculation. The results of the calculations are reported in Table 2.7.5-2.

Table2.7.5-1. Input values for the dietary burden calculation

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Residue definition for risk assessment in plants: **Trinexapac, free and conjugated (cereal grain, provisional)** **Trinexapac, free and conjugated, plus CGA300405 (cereal/grass feed items, provisional)**				
Wheat, straw	0.05	Median residue (tentative)[(a)]	0.17	Highest residue (tentative)[(a)]
Rye, straw*	0.05	Median residue (tentative)[(a)]	0.17	Highest residue (tentative)[(a)]
Barley, grain	0.15	Median residue (based on SEU data)	0.15	Median residue (based on SEU data)
Wheat, grain	0.08	Median residue	0.08	Median residue
Rye, grain*	0.08	Median residue	0.09	Median residue
Brewers’ grain	0.50	Median residue × default PF brewer’s grain (3.3)	0.50	Median residue × default PF brewer’s grain (3.3)
Distillers’ grain	0.26	Median residue × default PF brewer’s grain (3.3)	0.26	Median residue × default PF brewer’s grain (3.3)
Wheat gluten, meal	0.14	Median residue × default PF gluten feed meal (1.8)	0.14	Median residue × default PF gluten feed meal (1.8)
Wheat, milled byprods.	0.56	Median residue × default PF bran (7)	0.56	Median residue × default PF bran (7)

* - extrapolated from wheat

(a) Levels of trinexapac (free and consjugated) in straw are derived from combined dataset with major part of the samples not supported by storage stability. Conribution of metabolite CGA300405: not considered

Table 2.7.5-2. Results of the initial dietary burden calculation

	Intake (%)	Median dietary burden (mg/kg bw/d)	Maximum dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Residue definition for risk assessment in animals: **Poultry: trinexapac (free)** **Ruminants: trinexapac(free) plus metabolite CGA 113745, expressed as trinexapac**						
Cattle - Beef						
Wheat milled by-products	30	0.007	0.008	Wheat milled by-products	0.31	Y
Rye straw	20					
Barley grain	50					
Cattle - Dairy						

	Intake (%)	Median dietary burden (mg/kg bw/d)	Maximum dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Residue definition for risk assessment in animals: **Poultry: trinexapac (free)** **Ruminants: trinexapac(free) plus metabolite CGA 113745, expressed as trinexapac**						
Wheat milled by-products	30	0.01	0.011	Wheat milled by-products	0.30	Y
Rye straw	20					
Barley grain	40					
Sheep - Ram/Ewe						
Wheat milled by-products	40	0.011	0.017	Wheat milled by-products	0.40	Y
Rye straw	40					
Barley grain	20					
Sheep - Lamb						
Wheat milled by-products	50	0.017	0.018	Wheat milled by-products	0.41	Y
Rye straw	40					
Barley grain	10					
Swine - Breeding						
Wheat milled by-products	50	0.009	0.009	Wheat milled by-products	0.40	Y
Barley grain	50					
Swine -Finishing						
Wheat milled by-products	50	0.012	0.012	Wheat milled by-products	0.40	Y
Barley grain	50					
Poultry - Broiler						
Wheat milled by-products	20	0.017	0.017	Wheat milled by-products	0.25	Y
Barley grain	70					
Poultry - Layer						
Wheat milled by-products	20	0.018	0.018	Wheat milled by-products	0.27	Y
Wheat straw	10					
Barley grain	70					

	Intake (%)	Median dietary burden (mg/kg bw/d)	Maximum dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Residue definition for risk assessment in animals: **Poultry: trinexapac (free)** **Ruminants: trinexapac(free) plus metabolite CGA 113745, expressed as trinexapac**						
Poultry - Turkey						
Wheat milled by-products	20	0.015	0.015	Wheat milled by-products	0.21	Y
Barley grain	50					

The calculated dietary burdens for all groups of livestock were found to be above the trigger value of 0.004 mg/kg bw/d, therefore further investigation of residues in commodities of animal origin is necessary.

A guidance on fish is currently being elaborated (SANCO/11187/2013, Appendix J), however it has not been formally noted as a guidance document since several points need to be addressed (Standing Committees of 25-26 February 2013 and 22-23 April 2013). Consequently, no fish dietary burden has been calculated.

Poultry

According to the metabolism studies on poultry (see Volume 3 Section B.7.2.2.1), it is concluded that after exposure to the maximum dietary burden (about 50-57 times lower than the dose level of the metabolism studies), residue levels in poultry commodities are expected to remain below the enforcement LOQ of 0.01 mg/kg in tissues and eggs (only small amounts of trinexapac-ethyl equivalents/kg were found in egg white 0.0196 mg/kg, liver 0.013 mg/kg, skin 0.011 mg/kg and kidney 0.043 mg/kg). Hence, no livestock feeding study is needed.

Ruminants

No feeding studies on ruminants were submitted for renewal. However, ruminant feeding study assessed in the framework of the first Annex I inclusion have been included in the revised RAR. A livestock feeding study for trinexapac-ethyl on lactating cows was considered to be acceptable. The kidney was the only tissue of all samples analysed were a clear dose dependent increase of CGA 179500 residues was found. The residues in muscle and fat were below or around the LOQ of 0.02 mg/kg. In the liver the residue level was just above the LOQ only in the highest dose group. Residues in milk samples were only found in the highest dosed group, reaching 0.011 mg/kg. No detectable residues are expected in ruminant products at a nominal intake of CGA 179500 via feed (0.30-0.40 mg/kg feed calculated in Table 2.7.5-2). The available data are considered sufficient for deriving MRLs in ruminants. Significant residues in tissues and milk of ruminants are not expected and MRLs for these commodities can be established at the LOQ.

Pigs

The calculated maximum dietary intake of trinexapac residues for pigs is 0.012 mg/kg bw/d, which is lower than the one calculated for ruminants (Volume 1 Table 2.7.5-2). The metabolism of trinexapac-ethyl in the rat is not different from that in ruminants, therefore the feeding study on ruminants can be used to propose MRLs in pig products. Significant residues in tissues of pigs are therefore not expected and MRLs for these commodities can be established at the LOQ (0.01* mg/kg).

Fish

Fish feeding studies have not been conducted due to the lack of a guidance document. The TTF explanation is that: "Document SANCO/10181/2013 Rev. 2.1, 13 May 2013 states: "In some cases, agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, waiving of these particular data requirement points is considered acceptable as long as no test methods or guidance documents are published in the form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02."

Currently guidance for fish metabolism and fish feeding studies has not been finalized. It was noted in Section A.24 of the summary from the SCoPAFF meeting on 24 – 25 November 2014 that "the Commission working document is not yet finalized and ready to be noted as a guidance document." Additionally, "…the Commission emphasized that for the time being there are no agreed test guidelines and that hence the pertinent data requirements can be waived."

Additionally, the $\log P_{ow}$ is below 3 for trinexapac-ethyl.

Additional argumentation based on the lack of:

dietary burden calculation method, method for studying the nature of residues in fish; an agreed and practicable method for quantitatively studying the transfer of residues of concern into fish tissues was provided by the applicant and reported in Vol. 1 level 2.7.2. RMS considers the argumentation provided as not valid justification and believes that at least a dietary burden calculation as recommended in SANCO/11187/2013 should be submitted.

2.7.6 Summary of effects of processing

The effect of processing on the nature of trinexapac-ethyl and trinexapac was investigated in the framework of the peer review. Studies were conducted by Syngenta simulating representative hydrolytic conditions for pasteurisation (20 minutes at 90°C, pH 4), boiling/brewing/baking (60 minutes at 100°C, pH 5) and sterilisation (20 minutes at 120°C, pH 6). Two other studies were conducted by the members of the Task Force (Adama and Cheminova).

In the studies conducted by Syngenta and Cheminova, trinexapac was radiolabelled in the cyclohexane ring while the Adama study has been conducted with a different radiolabelled position (cyclopropane ring).

The Syngenta and Adama studies show that trinexapac degrades under elevated temperatures conditions, but represents the major part of the residue (~51-86% TRR). The major degradation products identified are CGA313458 (~4-21% TRR), CGA113745 (~10-12% TRR) and cyclopropane carboxylic acid (CGA224439) (~5-18% TRR), which haven't been found in the rat metabolism.

The Cheminova study shows that trinexapac remains stable under pasteurisation, baking/boiling/brewing and sterilisation conditions – which is different from the Syngenta and Adama studies.

It can be concluded that the nature of residues in processed commodities is different to the one in raw agricultural commodities.

As residues of trinexapac are expected to exceed 0.1 mg/kg in the RAC and as several degradates (>10 %TRR) were formed in the high temperature hydrolysis studies, investigation of the magnitude of residues in processed commodities has been conducted.

Processing studies of barley and wheat have been evaluated in the DAR 2003, but only trinexapac (free form) was measured in those studies. Eight studies were conducted in order to investigate the influence of processing of the residue in winter and spring barley after single application of trinexapac-ethyl (CGA 163935), but were considered not reliable and excluded from the assessment. One study was conducted in order to investigate the influence of processing of the residue in winter wheat after single application of trinexapac-ethyl (CGA 163935) at a rate of 0.2 kg as/ha.

Three additional studies on barley and wheat were conducted in 2006 and 2008; they measured the residue levels of trinexapac (free or free and conjugated) in flour and milling by-products.

New processing studies on barley and wheat have been conducted, in order to:

- mimic the representative processing conditions such as baking and brewing;
- measure trinexapac (free and conjugated) in raw agricultural commodities (RAC) and processed products;
- measure processing degradates CGA313458, CGA113745 and cyclopropane carboxylic acid (CPCA, also referred to as CGA224439).

The studies have been conducted at an elevated application rate (1×400 g a.s./ha) corresponding to 2N for barley and 3.2N for wheat.

Residue levels of trinexapac (free and conjugated) ranged from 0.5–2.8 mg/kg in wheat grain and from 1.56–1.9 mg/kg in barley grain. Residue levels in processed commodities were all above the LOQ, allowing derivation of robust processing factors.

Taking into account all the processing studies conducted on barley, it can be concluded, that residues of trinexapac (free) and CGA224439 in barley grain were concentrated in bran and brewers' yeast (TF>1). Residues of trinexapac (free and conjugated) were slightly concentrated in pearled barley (TF = 0.86 - 1.5) and barley bran (TF = 1.6 - 2.2), in one study and not concentrated in any of the processed fractions in another. Residues of metabolite CGA313458 were not concentrated in any of the processed fractions. Although results in barley bran and flour samples are not covered by storage stability data. Magnitude of CGA 313458 in above mentioned processed commodities and processing factors should be further assessed. Metabolite CGA113745 was not concentrated and only found in bran at the level of 0.01 mg/kg. Nevertheless all residue results are not covered by storage stability data and the metabolite is proven to be unstable. Residue levels of this metabolite in RAC and processed commodities as well as processing factors should be further assessed.

Taking into account all the processing studies conducted on wheat, it can be concluded, that residues of trinexapac (free) were concentrated in cleaned grain and total bran (TF 1.09-2.5), a slight concentration found in the wheat shorts and germ (TF 1.4 and 1.1 respectively). Residues of trinexapac (free and conjugated) were slightly concentrated only in cleaned grain (TF 1.1). Metabolite CGA313458 was concentrated in wholemeal bread (TF 1.5), but was not detected in any other fraction. As the results in wheat bran, flour and bread samples are not covered by storage stability data, magnitude of CGA 313458 in above mentioned processed commodities and processing factors should be further assessed. Residue of CGA224439 was concentrated in waste (offal), wholemeal bread and dry gluten (TF 1.45-2.00), and slightly concentrated in cleaned grain, total bran, wholemeal flour and germ (TF 1.10-1.21). Metabolite CGA113745 was not found in any fraction analysed. Nevertheless all residue results are not covered by storage stability data and the metabolite is proven to be unstable. Residue levels of this metabolite in RAC and processed commodities as well as processing factors should be further assessed when data on magnitude in RAC and processed commodities will be available. Samples of germ and middlings were not analysed for metabolites CGA313458 and CGA 113745 due to low sample weight.

At the expert meeting (PPR 171, 13 – 15 December 2017) the experts were unable to conclude on the relevant residues in processed commodities. To address the effect of food processing conditions on residues, four standard hydrolysis studies were submitted showing partially contradictory outcomes. Two studies were suggesting the stability of trinexapac-ethyl and trinexapac, respectively under hydrolysis conditions while the other two studies showed significant degradation under baking and sterilisation conditions. Further clarification by the applicant to explain the ambiguous findings in this standardised experiment is necessary.

Therefore a data gap was set - further clarification should be submitted by the applicant to explain the contradictory findings (stability vs. instability) in the standardised hydrolysis experiments.

2.7.7 Summary of residues in rotational crops

The metabolism of trinexapac-ethyl in rotational crops was investigated in lettuce, sugar beet, radish, winter wheat and corn using [^{14}C-cyclohexyl]-trinexapac-ethyl. One confined rotational crop study investigating the nature of residues following different plant-back intervals has been investigated during the peer review (lettuce, sugar beet, corn and wheat); a new study has been conducted in 2010 in order to cover a higher application rate (lettuce, radish and wheat).

The uptake of CGA 163935 in rotational crops, as analysed in original metabolism study on lettuce, winter wheat, sugar beets and corn after direct application of 0.15 kg as/ha radio-labelled compound to the soil, is very low (≤0.001 mg/kg). The application rate of CGA 163935 was 25% below the proposed GAP for barley (150 g instead of 200 g as/ha). N rate was lower and first plant back interval too long than recommended in OECD 502 (0.75 N and 99 days respectively). TRR was ≤ 0.001 mg/kg in all commodities, but circumstances of crop failure or closely rotated crops (7-30 DAT) were not assessed. This study was considered acceptable but not fully addressing metabolism of trinexapac-ethyl in rotational crops. For this reason a new rotational crop metabolism study was conducted (1.75 N rate and appropriate plant back intervals) fully addressing metabolism of trinexapac-ethyl in rotational crops.

In new rotational crop metabolism study, submitted for renewal, after one application of trinexapac-ethyl applied to bare ground at a rate of 0.33 kg a.s./ha (1.65 N the maximum rate of the representative crops (barley), the total radioactive residues in all RACs were very low < 0.01 mg/kg, except for some 30 day PBI foliage RACs (lettuce and wheat) were slightly above 0.01 mg/kg. However, no individual extractable ^{14}C-residue was found to be > 0.01 mg/kg for any RAC at any PBI. No extractable residue match parent. These finding suggest extensive and rapid soil degradation of parent and likely mineralization to CO_2, since little ^{14}C was take-up into any rotational crop. Results in both studies are comparable, as TRR were low and >0.01 mg/kg observed only at 30 days plant back interval.

Studies on the magnitude of trinexapac-ethyl residues in rotational crops are not required. Considering that in the above rotational crop metabolism study was carried out on a bare soil with 0.75N to 1.75N application rate, it can be concluded that trinexapac-ethyl residue levels in rotational commodities are not expected to exceed 0.01 mg/kg, provided that trinexapac-ethyl is applied in compliance with the representative GAP.

At the expert meeting (PPR 171, 13 – 15 December 2017) a data gap was set – to investigate the potential for uptake of residues bearing the cyclopropyl moiety in rotational crops and their identity.

2.7.8 Summary of other studies

No studies of residue levels in pollen and bee products were provided. TTF informed that residue study in honey will be available in first quarter 2018.

The data requirement objective of these studies is to determine the residue in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom.

In the Guidance Document on the risk assessment of plant protection products on bees (EFSA Journal 2013;11(7):3295), information about the relative attractiveness of different crops to honey bees, bumble bees and solitary bees is presented. For honey bees (which is of relevance to the potential transfer of residues from treated crops into edible bee products), in relation to wheat and barley the crops are not considered as being melliferous and are therefore not considered relevant for honey production. In addition, the crops are generally considered to be of low attractiveness to bees. Thus the potential for transfer of residues into honey is considered to be not significant for the ‘AIR’ representative crops and – given the levels of consumption of honey – of no concern for consumer safety.

In regard of this specific data requirement, Document SANCO/10181/2013 Rev. 2.1, 13 May 2013 states: “In some cases, agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, waiving of these particular data requirement points is considered acceptable as long as no test methods or guidance documents are published in the form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02.”

The applicants' current understanding is that there is no guidance yet finalised for assessing residue levels in pollen and bee product studies (suitable for human consumption assessment purposes). It is also noted in Section A.24 of the summary from the SCoPAFF meeting on 24 – 25 November 2014 that "the Commission working document is not yet finalised and ready to be noted as a guidance document." Additionally, "…the Commission emphasised that for the time being there are no agreed test guidelines and that hence the pertinent data requirements can be waived." Nevertheless the residue study in honey is currently ongoing.

2.7.9 Estimation of the potential and actual exposure through diet and other sources

Trinexapac ethyl

Based on the fully supported representative uses of wheat NEU&SEU and barley SEU, MRL application (rye) and animal commodities, the following estimate of dietary exposure through diet is calculated with EFSA PRIMo Model (rev-2), see also figure 2.7.9-1.

The toxicological profile of trinexapac-ethyl evaluated in the framework of Directive 91/414/EEC, resulted in an ADI being established at 0.32 mg/kg bw. An ARfD was then not deemed necessary. The same conclusion is reached during renewal process. As trinexapac was found in significant amounts in the rat (refer to Volume 3 CA B.6 for further details), the toxicological values of parent can be applied to trinexapac.

The chronic risk assessment (TMDI, theoretical maximum daily intake) is based on the calculated MRLs of SEU dataset for barley grain and combined NEU/SEU datasets for wheat grain (1.0 and 0.5 mg/kg, respectively), also rye grain (0.5 mg/kg, extrapolation from wheat) and edible animal products (0.01*mg/kg). As the definition for risk assessment for plant commodities is different to the one for enforcement, the MRLs for wheat, barley and rye grain have been multiplied by the conversion factor of 1.43 (see Volume 3 CA B.7.3 for further details).

The highest calculated value of the ADI is 2.3 % for DK child (see figure 2.7.9-1). The acute risk assessment is not necessary.

Chronic and acute exposure calculations for processing metabolite CGA224439 (CPCA) using TTC concept were provided by the applicant. Input values (STMR for wheat and barley grain) used in these calculations are different from the ones calculated by RMS. Residue definition for processed commodities is still open, therefore chronic and acute exposure for CPCA was not recalculated by RMS and was removed from Vol 1.

Trinexapac-ethyl			
Status of the active substance:	approved	Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0,32	ARfD (mg/kg bw):	n.a.
Source of ADI:	AIR3 RAR	Source of ARfD:	AIR3 RAR
Year of evaluation:	2016	Year of evaluation:	2016

Prepare workbook for refined calculations

Undo refined calculations

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

TMDI (range) in % of ADI minimum - maximum	
	2
No of diets exceeding ADI:	---

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
2,3	DK child	1,2	Wheat	1,0	Rye	0,0	Milk and cream,	
2,1	WHO Cluster diet B	1,9	Wheat	0,1	Barley	0,0	Rye	
1,7	WHO cluster diet D	1,5	Wheat	0,1	Barley	0,1	Rye	
1,5	IT kids/toddler	1,5	Wheat	0,0	Barley		FRUIT (FRESH OR FROZEN)	
1,4	WHO cluster diet E	0,9	Wheat	0,4	Barley	0,1	Rye	
1,3	WHO Cluster diet F	0,8	Wheat	0,3	Barley	0,2	Rye	
1,2	NL child	1,1	Wheat	0,1	Milk and cream,	0,0	Rye	
1,2	DE child	0,9	Wheat	0,2	Rye	0,0	Milk and cream,	
1,1	IE adult	0,6	Barley	0,5	Wheat	0,0	Rye	
1,0	ES child	1,0	Wheat	0,0	Milk and cream,	0,0	Meat, preparations of meat,	
1,0	UK Toddler	0,9	Wheat	0,1	Milk and cream,	0,0	Barley	
0,9	IT adult	0,9	Wheat	0,0	Barley		FRUIT (FRESH OR FROZEN)	
0,9	PT General population	0,9	Wheat	0,0	Rye	0,0	Barley	
0,8	WHO regional European diet	0,7	Wheat	0,1	Barley	0,0	Milk and cream,	
0,8	SE general population 90th percentile	0,7	Wheat	0,1	Rye	0,0	Milk and cream,	
0,8	ES adult	0,5	Wheat	0,2	Barley	0,0	Milk and cream,	
0,8	FR all population	0,7	Wheat	0,0	Milk and cream,	0,0	Meat, preparations of meat,	
0,7	FR toddler	0,6	Wheat	0,1	Milk and cream,	0,0	Meat, preparations of meat,	
0,7	UK Infant	0,6	Wheat	0,1	Milk and cream,	0,0	Meat, preparations of meat,	
0,7	NL general	0,5	Wheat	0,2	Barley	0,0	Milk and cream,	
0,6	DK adult	0,4	Wheat	0,2	Rye	0,0	Milk and cream,	
0,5	LT adult	0,2	Rye	0,2	Wheat	0,0	Barley	
0,5	UK vegetarian	0,5	Wheat	0,0	Milk and cream,	0,0	Barley	
0,4	FI adult	0,2	Wheat	0,2	Rye	0,0	Milk and cream,	
0,4	UK Adult	0,4	Wheat	0,0	Barley	0,0	Milk and cream,	
0,3	FR infant	0,2	Wheat	0,1	Milk and cream,	0,0	Meat, preparations of meat,	
	PL general population		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	

Conclusion:

The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of Trinexapac-ethyl is unlikely to present a public health concern.

Figure 2.7.9-1 Estimation of the potential exposure (chronic), EFSA PRIMo Model (rev-2)

Processing metabolite (CPCA) RMS comments

During the commenting period, EFSA stated:

For acute/chronic risk assessment of the processing metabolite CPCA, the TTC concept comparing predicted dietary exposure levels through processed products from cereals treated according to representative uses with a threshold (Cramer II, 1.5 μ/kg bw) is not acceptable. There is currently not implemented guidance on the subject tool and the metabolite is common to other a.s.

Also:

Since the EFSA PPR GD (2016) guidance on the residue definition is not yet adopted by Member States and the Commission, it cannot be used to assessment the risk for of dietary metabolites. To perform a comprehensive dietary risk assessment, the toxicological relevance and the magnitude of residues of CPCA in the relevant commodities for the representative uses should be established.

As a consequence, the applicant referred to JMPR, 2014, where a 90 day rat study on CPCA was reviewed for the active substance Aminocylopyrafor. An ADI in this report is proposed of 0.03 mg/kg bw /day. This endpoint is higher than that calculated by the conservative TTC approach. Though a 90-day rat study on CPCA (Carpenter C., 2012) is referred to in the JMPR review of active substance aminocyclopyrachlor (JMPR, 2014), it has not been submitted to the RMS for an independent assessment. Specific reference values in order to conduct a consumer risk assessment cannot be set (please refer to Vol. 1 Section 2.6.9.1)

RMS is of the opinion, that the risk assessment through diet for metabolite CPCA could not be finalised.

2.7.10 Proposed MRLs and compliance with existing MRLs

EU MRLs were reviewed under Article 12 of Regulation (EC) No 396/2005 in 2012 and formally placed on Annex II of the same regulation following Commission Regulation (EU) No 87/2014 of 31 January 2014. Since this time, several MRLs have been modified (Regulation (EU) No 2015/845).

Based on the representative uses of wheat and barley (barley SEU and wheat NEU&SEU are fully supported by data), the following MRLs are proposed, based on submitted data, see table below. The data presented in this document demonstrate that the proposed representative uses of trinexapac-ethyl will lead to exceedances of be within the newly modified MRLs for wheat and barley grain. The notifier has requested a modification of the existing EU MRL on rye. The data to support an increase of MRLs for this crop have been submitted to support the representative uses on barley and wheat. However calculated MRL from combined NEU/SEU dataset for wheat is 0.5 mg/kg, and no change is needed. Risk assessment calculations have been performed and do not lead to unacceptable risks to human health.

Table 2.7.10-1 Proposed MRLs (mg/kg)

Code	Commodity	Current MRL[a]	Proposed MRL

Code	Commodity	Current MRL[a]	Proposed MRL
0500010	Barley	3	1.0
0500070	Rye	0.5	0.5
0500090	Wheat	3	0.5
1010000	Animal tissues except kidney	0.01*	0.01*
-	Animal kidney	0.05	0.01*
1016030	Poultry liver	0.05	0.01*
1016050	Poultry edible offal	0.05	0.01*
1020000	Milk	0.01	0.01*
1030000	Birds eggs	0.01*	0.01*

** Indicates that the MRL is set at the limit of analytical quantification; [a] Commission Regulation (EU) No 2015/845 of 27 May 2015.*

2.7.11 Proposed import tolerances and compliance with existing import tolerances

No import tolerances have been proposed in the EU or applied for in any EU Member State.

2.8 Fate and behaviour in the environment

2.8.1 Summary of fate and behaviour in soil

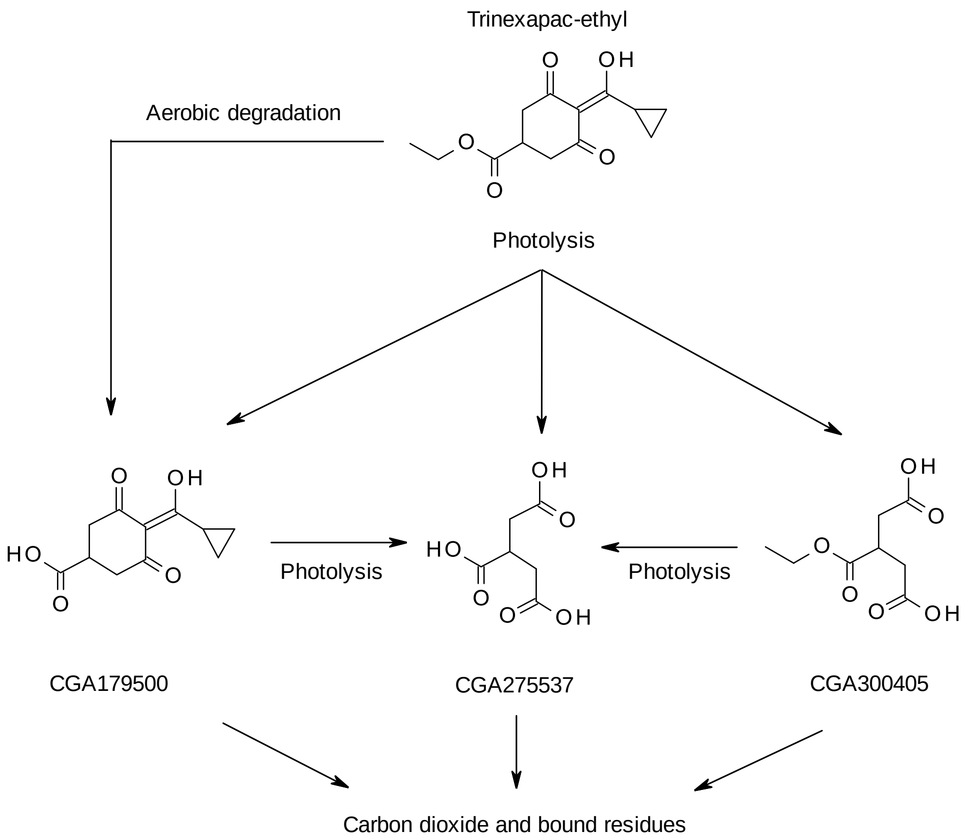
Route of degradation in soil

Aerobic degradation of trinexapac-ethyl was investigated in four studies at 20°C. Three different positions of ^{14}C-label were used: [3,5-^{14}C]cyclohexane[^{14}C]carboxylic acid ethyl ester]-, [cyclopropylhydroxy[^{14}C] methylene]- and [1,2,6-^{14}C]- labelled active substance. Trinexapac-ethyl readily degrades to CGA179500 in aerobic soil degradation studies, with levels up to 93% AR. CGA179500was subsequently mineralised to carbon dioxide and bound residues. No metabolites, other than CGA179500, have been detected over 5% AR. Mineralisation measured as volatile CO_2 ranged from 58% AR after 90 days, to 85% AR after 28 days.

Under anaerobic conditions in soil, dosed with the [cyclohexanedione-1,2,6-^{14}C]-labelled trinexapac-ethyl, CGA179500 was the major transformation product, with formation rate of 87% AR at 121 day, which in turn is stable to anaerobic conditions. Other metabolites were not detected at >5% of the applied radioactivity.

The potential for photolytic breakdown of labelled trinexapac-ethyl at the soil surface was carried out is moist and dry loam soil under artificial light. Under dry soil conditions metabolites CGA179500, CGA300405, and CGA275537 were reaching maximum mean amounts of 22.8%, 12.5%, and 10.8% of the applied activity, respectively. In moist soil conditions, CGA179500 and CGA275537 reached maximum mean amounts of 61.5% and 6.5% of the applied activity, respectively. Metabolite CGA275537 accounted for more than 5% of the amount of active substance added in two sequential measurements. In dark control samples only CGA179500 was measured at concentrations more than 10% of AR under dry and moist soil conditions. Therefore test results indicate that CGA300405 and CGA275537 are both photolysis metabolites, while CGA179500 may be formed by other reactions. The amount of radioactivity for the three degradates rose and declined concurrently, which would indicate that they all formed independently from the parent. However, after assessment of the chemical structures, it is not possible to rule out from the experiment that CGA275537 is not derived from CGA179500 or CGA300405. It will therefore be assumed that it could originate from trinexapac-ethyl, CGA179500 and CGA300405, as reflected in Figure 2.8.1-1.

Figure 2.8.1-1 Proposed degradation pathway of trinexapac-ethyl in soil



Rate of degradation in soil, laboratory studies

The new aerobic degradation studies as well as the previously submitted Spare (1992) study were evaluated using the FOCUS Kinetic Guidance (2006) to assess the degradation rate of trinexapac-ethyl and its metabolite CGA179500 in aerobic soil conditions. However, due to high percent of bound residues, DT_{50} values based on extractable trinexapac-ethyl and CGA179500 were considered unreliable for Hellstern (2008) and Walther (2008) aerobic degradation studies. Non-extractable residues for 18 Acres and Sarpy soils of Adams (2014) aerobic degradation study indicate that extraction method was insufficient for these soils and therefore following soils were excluded from the risk assessment. It was concluded that East Anglia, Capay and Gartenacker soils of Adams (2014) aerobic degradation study are appropriate for risk assessment purposes and DT_{50} calculation.

Under aerobic conditions, the persistence endpoints for trinexapac-ethyl in four soils tested varied from 0.013 to 0.72 days. For modelling purpose, first-order normalised DT_{50} values ranged from 0.045 to 0.72 days, with a geometric mean of 0.13 days.

Under aerobic soil conditions, the persistence endpoints in four soils tested for CGA179500 ranged from 1.0 to 32 days. For modelling purpose, first-order normalised DT_{50} values ranged from 1.0 to 39.5 days, with a geometric mean of 5.4 days.

The kinetic endpoints for photolysis metabolite CGA300405 were derived from metabolite applied study. The persistence endpoints ranged from 0.03 to 0.08 days while normalised first-order values for modeling purpose ranged from 0.11 to 0.52 days, with a geometric mean of 0.23 days.

The kinetic endpoints for photolysis metabolite CGA275537 were derived from metabolite applied study. The persistence endpoints and values for modeling purpose ranged from 0.17 to 0.27 days, with a geometric mean of 0.21 days.

Under anaerobic conditions the behaviour of non-extractable residues inquires the suitability of the study results to be used for substance rate calculation. Moreover, experiment involves a phase of aerobic period which could have triggered decomposition of trinexapac-ethyl before the anaerobic part of the study had started. Therefore the degradation rates reported in the study were not used for the risk assessment. Despite study deficiencies trinexapac-ethyl was not detected at the end of the study in all fourth soil types, what confirms that degradation occurs, but probably not as fast as reported. The major degradation product CGA179500 did not degrade under anaerobic conditions.

Rate of degradation in soil, field studies

As trinexapac-ethyl and CGA179500 do not trigger the need for field data, no new studies have been performed. Because of the deficiencies indicated in the field trials, studies available in the original DAR (2003) were not used for the risk assessment.

Assessment in relation to the P-criteria

The criteria for persistence in soil, as stated in Annex II to Regulation (EC) 1107/2009, are DT_{50} 120 days (PBT) and 180 days (POP and vPvB). All results for both trinexapac-ethyl and CGA179500 are clearly below these criteria. This is the case also for the photolysis soil metabolites CGA300405 and CGA275537.

Adsorption to soil

No new laboratory batch adsorption studies have been performed. Studies evaluated in original DAR were used and were considered appropriate. The adsorption of trinexapac-ethyl and its major metabolite CGA179500 were determined in fourth European soils. Adsorption and desorption of trinexapac-ethyl and CGA179500 were considered to be pH dependent due to the acidic moiety contained in both structures (lowest adsorption at high pH). Therefore, the worst case K_{FOC} values were chosen to be used in the risk assessment in accordance with the conclusions laid out in the Draft Assessment Report (Volume 3, Annex B, B.8, October 2003). The lowest K_{FOC} values of 60 and 145 L/kg were used for trinexapac-ethyl and CGA179500 respectively.

The mobility of photolytic metabolite CGA300405 was investigated in five European soils. Due to the high instability of the compound in soil, it was not possible to carry out a comprehensive adsorption/desorption study and determine mobility values for CGA300405 in soil using the batch equilibrium method. The only conclusion that can be drawn, that it is very unstable in soil : water system and is very mobile in soil. In the absence of reliable measurements lowest Kfoc value of 1mL/g, calculated with KOCWIN™ method, was used for the risk assessment.

The mobility of photolytic metabolite CGA275537 was investigated in five European soils. Sorption of CGA225537 is pH dependent. CGA225537 may be considered to exhibit from very high to low mobility with KFOC range from 4.35 L/kg to 1241.11 L/kg. The lowest KFOC value of 4.35 L/kg was used for the risk assessment.

Mobility in soil

Data to address this point were presented in the dossier submitted in 2003 for first inclusion in Annex I. No additional studies were submitted for the renewal. Column leaching studies are not required as reliable adsorption coefficient values for trinexapac-ethyl were obtained from the available adsorption and desorption study. Laboratory soil column leaching studies were performed with aged residues of trinexapac-ethyl in two soils. Koc could not be derived from this study, but minimal leaching of trinexapac-ethyl, CGA179500 or other metabolites was observed (radioactivity in leachate was 0.1-0.4% of that applied to the soil columns before leaching).

Lysimeter studies

Two field leaching studies were evaluated in the original DAR. No additional studies were submitted for the renewal. This study is not a data requirement in the Commission Regulation (EU) No 283/2013 setting the data requirements in accordance with Regulation (EC) 1107/2009.

Due technical deficiencies and low use rate used in the study, results were considered not appropriate for the risk assessment purposes and were provided as additional information only. Studies were carried out in Switzerland, using a suction lysimeter with probes at 80 cm and 120 cm depth capillary water was taken and analysed for CGA179500. Trinexapac-ethyl was applied in May 1993 at 125 g as/ha and May 1994 at 250 g/ha to wheat growing on a loamy sand soil (OM 1.8, pH 7.6) at Vouvray Switzerland. CGA179500 was not detected in soil water (sampled using suction cup samplers) at any time during the 329-days and 497-day sampling periods above the detection limit of 0.05 µg/L at 0.8 and 1.2 m depths.

2.8.2 Summary of fate and behaviour in water and sediment

Aerobic mineralisation in surface water

A surface water mineralisation study has been conducted to meet the new data requirement laid out in Commission Regulation (EU) No 283/2013. Trinexapac-ethyl mineralisation to CO_2 was low (did not exceed a 4% AR) and no other volatiles were detected (< 0.1% AR). Calculated DT_{50} values for trinexapac-ethyl in surface water were 21.2 - 25.9 days. In sterile system degradation was slower DT_{50} for trinexapac-ethyl was 69.9 days.

2.8.2.1 **Rapid degradability of organic substances**

Summary of relevant information on rapid degradability

Method	**Results**	**Key or Supportive study**	**Substance tested**	**Reference**
Aerobic mineralisation in surface water, OECD 309	Max DT_{50} = 25.9 days in fresh water	Key	^{14}C-trinexapac-ethyl	Volkel W., 2014
Water/sediment study, BBA IV (5-1, 1990) Guidelines and Pesticide Assessment Guidelines, Subdivision N. (1982)	Max DT_{50} = 5 days in water for trinexapac-ethyl, Max DT_{50} = 18 days in whole system for trinexapac acid	Key	^{14}C-trinexapac-ethyl	Muller-Kallert, H.M., 1993

Assessment in relation to the P-criteria

Following criteria for persistence in water and sediment are stated in Annex II to Regulation (EC) 1107/2009:

- DT_{50} in water: POP – 60 days, PBT – 40 days (fresh) and 60 days (marine), vPvB – 60 days (all water)
- DT_{50} in sediment: POP – 180 days, PBT – 120 days (fresh) and 180 days (marine), vPvB – 180 days (all sediment)

Data on fate and behaviour of trinexapac-ethyl or its metabolites in marine water or sediment is not available. For trinexapac-ethyl longest DT_{50} of 25.9 days observed in fresh water in aerobic mineralisation study. For trinexapac-ethyl maximum DT_{50}, in water was 5.0 days. For CGA179500 maximum DT_{50} in whole system was 18 days.

Adsorption to sediments is minimal with levels not being observed above 6.9% of AR for both trinexapac-ethyl and CGA179500 therefore DT_{50} values in fresh water sediments are not available.

Therefore available study results for both trinexapac-ethyl and CGA179500 are below P-criteria.

2.8.2.1.1 Ready biodegradability

The following study was evaluated in the original DAR of trinexapac-ethyl in 2003 (Baumann, W., 1993). Study was performed to determine the biodegradability of trinexapac-ethyl (purity 94.5%) in a carbon dioxide evolution test in activated sludge in accordance with the Guideline 92/69/EEC C.4-C, ready biodegradability carbon dioxide evolution test. Test was performed in duplicate with test media containing 26.9 and 27.9 mg test substance/L, equivalent to 16.6 and 17.2 mg theoretical organic carbon/L. Test was performed in 2 litre flasks which were connected to CO2 traps. A reference substance of 15 mg DOC/L and a water control were included in the experiments. Measurements of the CO2 content as inorganic carbon were performed with a carbon analyzer on the days 0, 3, 6, 8, 10, 15, 20, 24, 28 and 29.

Biodegradation of the test substance was 10% after 29 days and biodegradation of the reference was 87% after 29 days.

2.8.2.1.2 In respect to study results trinexapac-ethyl is classified as not readily biodegradable. BOD5/COD

Data not available.

2.8.2.2 **Other convincing scientific evidence**

2.8.2.2.1 Aquatic simulation tests

Data not available.

2.8.2.2.2 Field investigations and monitoring data (if relevant for C&L)

Data not available.

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

Data not available.

2.8.2.2.4 Water and sediment degradation data

Aerobic water/sediment study was conducted with ^{14}C-trineaxpac-ethyl in two water/sediment systems: one with Rhine water (pH8.2) and sand sediment, and another with pond water (pH8.5) and loam sediment. CGA179500 in both systems, the maximum formation rate in water was 48% and 64% in the pond and river systems respectively. No other metabolites were detected in water/sediment systems above 5% AR. Adsorption to sediments is minimal with levels not being observed above 6.9% of AR for both trinexapac-ethyl and CGA179500. Partitioning to sediment might be greater than this under more acidic test conditions, due to the pH dependence of adsorption. However Notifier stays that acidic conditions are not relevant for European surface water bodies. Further data to clarify this point were not provided.

The degradation rates of trinexapac-ethyl and CGA179500 have been reassessed following to current guidance and have been determined according to the latest update of the FOCUS Kinetic Guidance (2006). In water/sediment systems, trinexapac-ethyl rapidly degrades to CGA179500 in the water column, which in turn mineralises to carbon dioxide and bound residues.

For trinexapac-ethyl, the single first order DT_{50}, water were 3.3 and 5.0 days (average 4.0 days) and the single first order DT_{50}, whole system were 3.7 and 5.1 days (average 4.4 days). For CGA179500 the single first order DT_{50} in whole system were 14 and 18 days (average 16 days).

2.8.2.2.5 Hydrolysis

New hydrolysis study (Adam, 2015) on trinexapac-ethyl has been submitted to the existing hydrolysis studies (Spare 1990d and Spare 1992b). The active substance is stable at pH 7 and quickly hydrolyses to CGA179500 under basic conditions (pH 9) with half-life values of 7.2 and 11.3 days. Under acidic conditions, trinexapac-ethyl slowly hydrolyses with half-life values of 514 and 221 days at pH 5 and 188 days at pH 4 at 25°C.

SYN549299 forms up to 23% AR after 64 days at pH = 4 and 24.7°C. At pH 5 and 25°C, degradation was to CGA179500 and mono-ethyl ester of tricarboxylic acid, which were observed up to 18% AR and 12.5% of AR respectively after 179 days.

Applicant stays, that pH value at which SYN549299 was detected (pH = 4) is not representative of the majority of surface waters found in Europe. A 2012 dataset of European water bodies indicates that 95% of lakes and 99% of rivers have their average pH above 6. Therefore it was assumed that SYN549299 will not form at levels of concern in the environment and were not considered further in the assessment.

Two new hydrolysis studies on CGA179500 are presented. Study results indicate that CGA179500 is hydrolytically stable under neutral and alkaline conditions (pH 7 and pH 9). However, under acidic conditions, three metabolites have been observed over 10% AR: CGA113745 forms up to 18.6% AR (pH 4), CGA313458 forms up to 36.8% AR (pH 4) and CGA224439 (not unequivocally identified) up to 35% (pH 5).

New data helped to characterise the unidentified metabolite found above 10% AR in the Mamouni (2002) study, were two major degradates were formed over 10% AR: CGA313458 reached levels up to 31.4% AR (at pH 4) and unknown metabolite was not identified and reached levels up to 34.6% AR (at pH 5). Based on different radiolabelling pattern used in the studies and on analysis of hydrolytic pathway of CGA179500, unknown metabolite found in Mamouni study (2002) was attributed to CGA224439.

It was raised in the EFSA conclusion (EFSA Scientific Report (2005) 57, 1-70) that "Member States may need to require further information to address the nature of residues that might occur in acidic surface water bodies, and if the presence of novel breakdown products under these conditions is confirmed, further information to complete an aquatic risk assessment". New data to clarify this point were not provided by Notifier. Applicant stays, that it is unlikely that CGA113745, CGA313458 and CGA224439 will form at levels of concerns in the water column as mineralisation to carbon dioxide is the major route of CGA179500 degradation in European surface water bodies but also that there is a very low probability that CGA179500 will be exposed to acidic conditions. These degradation products were not considered further in the risk assessment.

2.8.2.2.6 Photochemical degradation

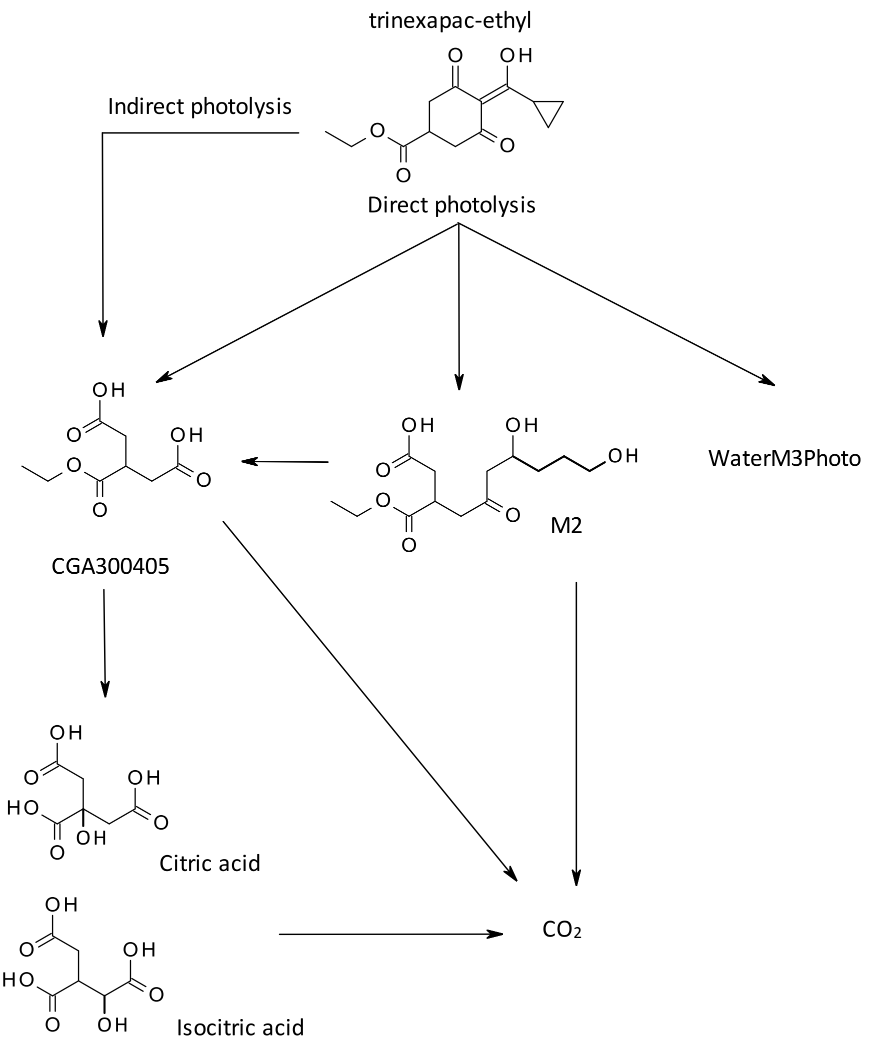
Previously submitted photolysis studies showed technical deficiencies therefore two new studies are submitted in sterile and natural buffered water under artificial light.

In sterile water trinexapac-ethyl is readily degraded with half-life value of 5.4 days (natural light, 50ºN). Following metabolites were observed above 10% AR: CGA300405 maximum occurrence of 41.2% of AR and still rising at the end of the study, 3 carboxylic acid ethyl ester-7-hydroxypropyl-5-oxo,7-hydroxyheptanoic acid (M2), reaching a maximum of 17.9% AR at day 5 and Water M3Photolysis (structural isomer of the parent), reaching a maximum of 16.9% AR at day 5.

In natural water at irradiation equivalent to sunlight at latitude of 35°N, the $t_{1/2}$ (SFO) was 15.3 days for trinexapac-ethyl. One major photodegradate CGA300405 was produced, this continually increased throughout the irradiation period reaching a maximum of 83.4% of applied radioactivity by day 7.

In natural water at irradiation equivalent to sunlight at latitude of 30°N two degradates were observed over 10% AR: CGA300405 up to 61% AR and citric acid (or isocitric acid) up to 11% AR. Citric acid and/or isocitric acid was observed in a protocol that had major technical deficiencies and was not conducted up to the current guidelines (irradiation time and number of samples analysed). Therefore citric acid and/or isocitric acid were not considered further in the risk assessment. In Figure 2.8.2-1, the proposed route of degradation for trinexapac-ethyl in the direct and indirect photolysis studies has been summarised.

Figure 2.8.2-1 Proposed photolytic degradation pathway of trinexapac-ethyl in direct and indirect photolysis



2.8.2.2.7 Other / Weight of evidence

Non

2.8.3 Summary of fate and behaviour in air

Trinexapac-ethyl has a low volatility of 2.16 x 10-3 Pa at 25 ºC and is shown to have insignificant volatilisation from soil. Volatilisation from plant surfaces was up to 10 – 15%, based on laboratory studies at 20ºC over 24 hours. The atmospheric indirect photolytic oxidation half-life for trinexapac-ethyl was estimated by the Atkinson method of calculation to be 1.29 – 10.8 hours and 3.2 – 3.9 hours for trinexapac acid. The trinexapac-ethyl that volatilises from plant surfaces would therefore not be expected to be subject to long range transport in the atmosphere.

2.8.3.1 **Hazardous to the ozone layer**

Table 70: Summary table of studies on hazards to the ozone layer

Method	Results	Remarks	Reference
Atkinson method	Trinexapac-ethyl DT_{50} = 1.29 –	Calculations	Stamm, E., 1999

Method	Results	Remarks	Reference
	10.8 hours		
Atkinson method	CGA179500 DT_{50} = 3.2 – 3.9 hours	Calculations	Palm, W.U., 1993b

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

Trinexapac-ethyl is quickly degraded in air therefore long range transport of trinexapac-ethyl in air is consequently unlikely. Due to the low volatility and rapid photochemical oxidative degradation in air of trinexapac-ethyl; local and global effects are expected to be negligible.

2.8.3.1.2 Comparison with the CLP criteria

The substance is not mentioned in Annexes of the Montreal Protocol.

2.8.3.1.3 Conclusion on classification and labelling for *hazardous to the ozone layer*

Not classified.

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

No monitoring studies were conducted as part of the new submission.

2.8.5 Definition of the residues in the environment requiring further assessment

Compartment	Residue	Justification
Soil	Trinexapac-ethyl	Parent, by default
	CGA179500	>10%, major degradation product in soil
	CGA300405	>10% in soil photolysis
	CGA275537	>10% in soil photolysis
Groundwater	Trinexapac-ethyl	Parent, by default
	CGA179500	>10%, major degradation product in soil
	CGA300405	>10% in soil photolysis
	CGA275537	>10% in soil photolysis
Surface water and Sediment	Trinexapac-ethyl	Parent, by default
	CGA179500	>10%, major degradation product in soil >10% in water/sediment
	CGA300405	>10% in soil photolysis >10% in water photolysis
	M2 (3-carboxylic acid ethyl ester-7-hydroxypropyl-5-oxo,7-hydroxyheptanoic acid)	>10% in water photolysis
	WaterM3Photolysis (structural isomer of the parent)	>10% in water photolysis
	CGA275537	>10% in soil photolysis
Air	Trinexapac-ethyl	Parent, by default

2.8.6 Summary of exposure calculations and product assessment

PECsoil

Acceptable PECsoil were presented for the representative uses in winter cereals. The scenario with 200 g a.s./ha at BBCH 25 assuming 20% crop interception results in the highest PECsoil. All PECsoil values were calculated using standard equation from FOCUS (1997) and default soil depth of 5 cm and bulk density of 1.5 g/cm.

PEC values in soil were calculated for trinexapac-ethyl, its major soil metabolites CGA179500 and soil photolysis metabolites CGA300405 and CGA275537. Initial PECsoil as well as short and long term PEC were presented. For metabolites pseudo application rate was considered with parent applied dose corrected with maximum occurrence and molar ratio.

For trinexapac-ethyl representative worst case laboratory DT_{50} of 0.72 days was used for estimation PEC values in soil. For CGA179500 normalised but not moisture content maximum laboratory value of 53 days, representing the worst-case, was used for estimation PEC values in soil. For photolysis metabolites CGA300405 and CGA275537 maximum laboratory values of 0.52 days and 0.27 days correspondingly were used for estimation PEC values in soil.

PECgroundwater

The estimation of PEC_{GW} for trinexapac-ethyl and its soil metabolites has been carried out according to current guidance requirements. The models FOCUS-PEARL 4.4.4, FOCUS-PELMO 5.5.3 and MACRO 5.5.4 were used to simulate the leaching behaviour of trinexapac-ethyl and its metabolites.

PECgw calculations were performed for representative use considering all FOCUS groundwater scenarios that are parameterized for winter and spring cereals. Crop interception values of 20% were considered. Applications were considered to start at the earliest growth stage covered by the GAP.

Since the degradation of trinexapac-ethyl in soil can be attributed to microbial and photolytic contributions, two separate leaching assessments were presented. One simulates the potential of trinexapac-ethyl and CGA179500 originating from the microbial contribution of the soil degradation, to leach to groundwater. The other assessment simulates the potential of CGA300405 and CGA275537, originating from the photolysis contribution in soil, to leach to groundwater. Since for metabolites CGA300405 and CGA275537 reliable kinetic degradation pathway was not established, a pseudo application rate was derived from the parent application rate, the maximum occurrence established in the photolysis experiment and adjusted for the molecular weight.

Following annual application of trinexapac-ethyl to winter and spring cereals at 200 g a.s./ha per year, the overall maximum PEC_{GW} in leachate at 1 m soil depth does not exceed 0.001 µg/L for the parent and metabolites CGA179500, CGA300405 and CGA275537.

PECsurface water and PECsediment

The estimation of PEC_{SW} and PEC_{SED} for trinexapac-ethyl, CGA179500, CGA300405 and CGA275537 were performed using FOCUS Step 1 and 2 models, version 3.2.

Use of 200 g a.s./ha in winter and 150 g a.s./ha in spring cereals were simulated. The crop interception was set to 'intermediate canopy' (equivalent to 20% interception) for trinexapac-ethyl, CGA179500 and CGA300405. For CGA275537 "no drift" option was set. Calculations were performed for all available Step 2 scenarios – i.e. North and South Europe and all three seasons.

The estimation of PEC_{SW} and PEC_{SED} for the metabolites M2 and WaterM3Photolysis were derived from the concentrations of the active substance based on maximum occurrence in water and adjusted for the molecular weight.

The complete PECsw calculations are presented in Document CP, Section 8.

2.9 Effects on non-target species

2.9.1 Summary of effects on birds and other terrestrial vertebrates

Avian acute oral and long – term reproduction studies have been carried out with trinexapac-ethyl. 3 acute avian studies were available, however one endpoint was not considered reliable. Therefore, the lowest available endpoint for the Mallard duck of LD50 >2000 mg a.s./kg bw is used in the avian acute risk evaluation. On the basis of 2 reproductive studies an avian NOEL of 17.6mg a.s./kg bw/d was set as the lowest available endpoint.

Mammalian acute oral and long-term reproduction studies have been carried out with trinexapac-ethyl and with A8587F equivalent formulation A8587B. From the section B.6 on Mammalian toxicology, the acute and reproductive endpoints were derived for the ecotoxicological risk assessment. The lowest available endpoins for rat of LD50 4210 mg a.s./kg bw and >750 mg a.s./kg bw (derived from study with formulation) were used in the mammalian risk assessment. For defining an ecotoxicologically relevant NOAEL detailed consideration of an available 6 long-term, reproduction and teratogenicity studies with mammalian species was made (See Volume 3, (CP) B.9.1.2). An overall mammalian NOAEL for rabbit was concluded to be 60 mg a.s./kg bw.

2.9.2 Summary of effects on aquatic organisms

2.9.2.1 Bioaccumulation

Table 71: Summary of relevant information on bioaccumulation

Method	Species	Results	Key or Supportive study	Remarks	Reference
Test freely adapted after: Subpart N, Environmental Chemistry Guideline Reference No. 165-4 and Laboratory Studies of Pesticide Accumulation in Fish (1982).	Bluegill sunfish (*Lepomis macrochirus*)	BCF is 6 L/kg wwt for whole fish tissue Uptake/depuration kinetics BCF is 100% after 14 days		BCFs in *Lepomis macrochirus* were 6 L/kg wwt for whole fish, 2.5 L/kg wwt for edible parts and 11 L/kg wwt for non-edible parts. Trinexapac-ethyl was demonstrated to have a low BCF in bluegill	CGA163935/0174 Anonymous, 1990 CA8.2.2.3/01
Test freely adapted after: FIFRA 165.4	Bluegill sunfish (*Lepomis macrochirus*)	BCF is 3.5 L/kg wwt for whole fish tissue		Accumulation potential in aquatic non-target organisms is hence considered to be low	CGA163935/0175 Anonymous, 1991 CA8.2.2.3/02
OECD 117 Shake flask method	-	Octanol-water partition coefficient, $LogP_{ow}$ = -0.29		Accumulation potential in aquatic non-target organisms is	Kettner R, 1999 Study no. 77863 (KCA 2.8/01)

				hence considered to be low	

2.9.2.1.1 Estimated bioaccumulation

The octanol - water partition coefficient of trinexapac-ethyl is pH-dependent and at environmentally relevant pH-values of approximately 7, trinexapac-ethyl has a log Pow below 3 (pH 6.9 log Pow = -0.29). Similarly the metabolite CGA179500 (trinexapac) has a log Pow of below 3 (pH 1.8 log Pow = 1.8 and decreasing at higher pH). These log Pow values are lower than the EU trigger of 3, indicating that the potential for accumulation in aquatic non-target organisms is low, therefore no further assessment is necessary. However, there are two studies previously submitted in the EU review where the potential for bioaccumulation has been measured (see below).

2.9.2.1.2 Measured partition coefficient and bioaccumulation test data

The partition coefficient for trinexapac-ethyl was measured via shake flash method to be log Pow =-0.29 at pH 6.9. The values in the study report (Kettner, R. (1999)) do not indicate a potentiality for bioaccumulation.

A bioconcentration study with ^{14}C-ring-labeled trinexapac-ethyl (radiochemical purity: 96.2%) was performed (Anonymous, (1990) (CA8.2.2.3/01)). Water quality parameters were within accepted range. During the 28 d of exposure, actual concentrations in the test solution were 1.4 ± 0.1 mg/L. During depuration, actual concentrations were ≤ 0.15 mg/L.

1.4 g/L: Steady state (based on r.a.) was reached after 28 days (by graph) for the BCFs based on wet weight whole organism (after 3 days; mean steady state concentration: 8.6±2.5 mg/kg), wet weight edible (after three days; mean steady state concentration: 3.6 ± 0.1 mg/kg) and inedible parts (after ten days; mean steady state concentration: 15 ± 5.8 mg/kg). Depuration DT_{50} 1 - 3 days (whole fish; all tissue portions). After seven days of depuration all ^{14}C-residues in the fish on the last day of exposure, had been eliminated from the whole body tissues.

CT_{50} Tissue concentration was below the detection limit. 6.5% of the accumulated ^{14}C-residues in edible tissues was extractable with hexane, after 28 d of exposure, and 45% of the accumulated ^{14}C-residues in edible tissues was extractable with acetonitrile (32% not extractable). After 28 days, 85% of the ^{14}C-residues in water were determined as trinexapac-ethyl.

Residues during exposure								
Part of fish	Residues in mg trinexapac-ethyl equivalents/ kg fish, fresh weight after:							
	0d*	1d	3d	7d	10d	14d	21d	28d
Edible	≤ 3.7	2.7	3.5	3.7	3.4	3.5	3.7	3.8
Non-edible	≤ 3.8	12	12	17	13	13	19	17
Whole fish	NA	6.5	7.2	9.5	7.8	7.4	10	9.8

d: day. *-control fish, background values. NA- not applicable, these values could not be calculated

Residues during depuration					
Part of fish	Residues in mg trinexapac-ethyl equivalents/ kg fish, fresh weight after:				
	1d	3d	7d	10d	14d
Edible	3.6	1.3	< 1.9	< 1.0	< 1.4

Non-edible	12	2.4	< 1.9	< 1.6	< 1.6
Whole fish	7.1	1.8	NA	NA	NA

d: day. NA- not applicable, these values could not be calculated

Conclusion

CGA-163935 was demonstrated to have a low BCF in bluegill. The BCFs (based on total radioactive residue) of 2.5, 11 and 6 were calculated in edibles, non-edibles and the whole fish, respectively. CGA-163935 depurated rapidly from all tissues, with a half-life between 1 and 3 days. After 28 days, 85% of the ^{14}C-residues in water were determined as trinexapac-ethyl. No characterisation of (^{14}C-) residues, apart from the ^{14}C-residues in water after 28 days of exposure. Bioconcentration factors are based on total radioactivity and do not necessarily refer to trinexapac-ethyl.

The validity criteria of OECD Test Guideline 305 are met:

- The water temperature variation was less than ± 2°C. (Reported range 17.0 – 17.5).
- The concentration of dissolved oxygen did not fall below 60% saturation (6.4 mg/L at 17.5°C equates to 67.3% ASV).
- The concentration of the test substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase (Range 1.26 – 1.88 mg/L and all are within this).
- The concentration of the test substance is below its limit of solubility in water. (The water solubility of 21.1g/L for trinexapac-ethyl. Tested concentration = 1.4 mg/L)
- The mortality or other adverse effects/disease in both control and treated fish is less than 10% at the end of the test (during both the exposure and depuration periods only two mortalities occurred in the solvent control aquarium (none in the treatment or depuration aquaria)).

Consequently, this study is still considered valid and acceptable for use in the risk assessment.

As this study was performed in accordance with the procedures described in Subpart N, Environmental Chemistry Guideline Reference No. 165-4 and Laboratory Studies of Pesticide Accumulation in Fish (1982), the lipid content of fish tissue was not measured.

A second bioconcentration study with ^{14}C-trinexapac-ethyl (label in the ring) was performed. (Anonymous, (1991) (CA8.2.2.3/02)). Water quality parameters were within accepted range. During the 28 d of exposure, actual concentrations in the water were 1.42±0.1 mg/L. The concentrations of ^{14}C-residues in edible tissues were 3.02 and 3.2 mg/kg wwt, after 14 and 28 days, respectively. The concentrations of ^{14}C-residues in inedible tissues were 59.39 and 36.33 mg/kg wwt, after 14 and 28 days, respectively. Extracted tissue fractions showed predominantly parent trinexapac-ethyl (c. 48% of AR after 14 days and c. 42% after 28 days) and its metabolite trinexapac (CGA 179500) (also c. 48% of the radioactivity after 14 days and c. 42% after 28 days). In inedible tissue, the trinexapac was the major component (trinexapac: 78.6% after 14 days, and 75.6% after 28 day; trinexapac-ethyl: 5.0% after 14 days, and 6.1% after 28 days). In the edible tissue, also small amounts of two polar substances were measured (‘peak A’: 2.7-3.4% and ‘peak B’: 2.5-4.7%). These polar substances were also found in inedible tissue (in small amounts). These determinations were confirmed by HPLC, FAB/MS and

GC/MS. Further analysis showed that ‘peak A’ contained multiple components, and that ‘peak B’ referred to 6-cyclopropyl-6-hydroxy-2-methyl-4-oxo-hexa-2,5-dienoic acid.

Residues during exposure			
Part of fish	Residues in mg trinexapac-ethyl equivalents/ kg fish, fresh weight after:		
	14d	28d*	28d
Edible	2.67	1.82	2.05
Non-edible	14.0	10.9	8.85
Whole fish	7.84	5.94	5.04

d: day. *-This goup of fish had been exposed from day 14 through day 28 of the exposure

Conclusion

It should be noted that the BCF for the edible parts refers to a mixture of trinexapac-ethyl and its major metabolite trinexapac, whereas the BCF for non-edible parts predominantly refers to trinexapac.

It should be noted that the BCF for the edible parts refers to a mixture of trinexapac-ethyl and its major metabolite trinexapac, whereas the BCF for non-edible parts predominantly refers to trinexapac.

The BCFs (based on total radioactive residue) of 1.9, 9.9 and 5.5 were calculated in edibles, non-edibles and the whole fish, respectively, after 14 days and of 1.4, 6.2 and 3.5 were calculated in edibles, non-edibles and the whole fish, respectively, after 28 days.

The bioconentration of trinexapac-ethyl was demonstrated to be negligible, with BCFs ranging from 1-10.

The validity criteria of OECD Test Guideline 305 are met:

- The water temperature variation was less than ± 2°C. (Reported range 17 – 18°C).
- The concentration of dissolved oxygen did not fall below 60% saturation (measured: 73-83%).
- The concentration of the test substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase. (Mean conc. of test item 1.42 mg/L ± 0.1 mg/L)
- The concentration of the test substance is below its limit of solubility in water. (The water solubility of 21.1g/L for trinexapac-ethyl. Tested concentration = 1.4 mg/L)
-
- The mortality or other adverse effects/disease in both control and treated fish is less than 10% at the end of the test (there were two mortalities among a total of 1500 fish exposed).

Consequently, this study is still considered valid and acceptable for use in the risk assessment.

As this study was performed in accordance with a test freely adapted after FIFRA 165.4, the lipid content of fish tissue was not measured. Consequently, it is not possible to express the bioconcentration factor as a function of the lipid content of the fish.

An applicant has provided such information:

The bioconcentration factors were only measured for edible, non-edible and whole body tissue in these old studies (Anonymous, 1990 and Anonymous, 1991 (CA8.2.2.3/01 and CA8.2.2.3/02)) done according to the protocol at that time. However both studies are with *Lepomis macrochirus* which typically has a lipid content of around 5%, and the BCFs were calculated with an assumed lipid content of 5%. Even if lipid values of 1 or 10%

were used there would still be very little change in the resultant BCFs. From these data where the measured BCFs ranged from 1.3 to 11 there are no indications of concerns from bioaccumulation. The observed values are all well below any regulatory trigger values.

2.9.2.2 Acute aquatic hazard

Table 72: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference
US EPA/FIFRA Guideline 72-1	Rainbow trout (*Oncorhynchus mykiss*)	trinexapac-ethyl (purity 96.6%)	96 h-LC_{50} 68 mg a.s./L (nom)			Anonymous, (1990) CA8.2.1/01 CGA163935/0014
US EPA/FIFRA Guideline 72-1	Bluegill sunfish (*Lepomis macrochirus*)	trinexapac-ethyl (purity 96.6%)	96 h-LC_{50} >130 mg a.s./L (nom)			Anonymous, (1990a) CA8.2.1/02 CGA163935/0015
US EPA/FIFRA Guideline 72-1	Common carp (*Cyprinus carpio*)	trinexapac-ethyl (purity 92.2%)	96 h-LC_{50} 57 mg a.s./L (nom)			Anonymous, (1991) CA8.2.1/03 CGA163935/0163
US EPA/FIFRA Guideline 72-1	**Channel catfish (*Ictalurus punctatus*)**	**trinexapac-ethyl (purity 92.2%)**	**96 h-LC_{50} 35 mg a.s./L (mm)**	**Key**	**35 mg a.s./L**	**Anonymous, (1991a) CA8.2.1/04 CGA163935/0164**
EPA guideline No. 72-3	(Sheepshead minnow) *Cyprinodon variegatus*	trinexapac-ethyl (purity 92.2%)	96 h-LC_{50} 180 mg a.s./L (mm)			Anonymous, (1991b) CA 8.2.1/05 CGA163935/10635
US EPA/FIFRA Guideline 72-2	*Daphnia magna*	trinexapac-ethyl (purity 96.6% a.s.)	48 h-LC_{50} >142.5 mg a.s./L (nom)			Smith et al. (1990) CGA163935/16
EPA Guideline No. 72-3	Eastern oyster (*Crassostrea virginica*)	trinexapac-ethyl (purity 92.2%)	Shell deposition 96 h-LC_{50} 89 mg a.s./L (mm)			Dionne (1991) CGA163935_10636
EPA Guideline No. 72-3	**Bay shrimp (*Mysidopsis bahia*)**	**trinexapac-ethyl purity 92.2%)**	**96 h-LC_{50} 6.5 mg a.s./L (mm)**	**Key**	**6.5 mg a.s./L**	**Sousa (1991) CGA163935_10634**

2.9.2.2.1 Acute (short-term) toxicity to fish

Acute fish toxicity tests were conducted in four freshwater and one marine species. All studies were considered to be relevant, reliable and adequate for classification purposes.

The sensitivity of *Oncorhynchus mykiss* (Rainbow trout) to trinexapac-ethyl was determined in a GLP-compliant semi static test performed to US EPA/FIFRA Guideline 72-1 (Anonymous, 1990 (CA8.2.1/01)). The 96-hour LC_{50} for *O.mykiss* was determined to be 68 mg a.s./L (95% confidence interval 58.6 - 79.0 mg trinexapac-ethyl/L). The NOEC (*Oncorhynchus mykiss*, 96h) = 29 mg trinexapac-ethyl/L. The results are based on the nominal test substance concentrations. Analytical results: The levels of trinexapac-ethyl were measured in the low, middle and highest exposure solutions on each day of the 4 day test. The sample detection limit was 0.2 mg/L, and the mean measured values ranged from 93% to 103% of the nominal values. Validity criteria were met in accordance with OECD guideline 203 (1984): Control mortality was 0% and dissolved oxygen was retained above 60% saturation.

The sensitivity of *Lepomis macrochirus* (Bluegill Sunfish) to trinexapac-ethyl was determined in a GLP-compliant semi static test performed to US EPA/FIFRA Guideline 72-1 (Anonymous, 1990a (CA8.2.1/02)). The 96-hour LC_{50} for *L. macrochirus* was determined to be 130 mg a.s./L (95% confidence interval not applicable because the LC_{50} value was greater than the highest concentration tested). The NOEC (*L. macrochirus*, 96h) = 46.6 mg trinexapac-ethyl/L. The results are based on the nominal test substance concentrations. Analytical results: The levels of Trinexapac-ethyl were measured in the low, middle and highest exposure solutions on each day of the 4 day test. The sample detection limit was 0.2 mg/L, and the mean measured values ranged from 96% to 97% of the nominal values. Validity criteria were met in accordance with OECD guideline 203 (1984): Control mortality was 0% and dissolved oxygen was retained above 60% saturation.

The sensitivity of *Cyprinus carpio* (Common carp) to trinexapac-ethyl was determined in a GLP-compliant flow-through test peformed to US EPA/FIFRA Guideline 72-1 (Anonymous, 1991 (CA8.2.1/03)). The 96-hour LC_{50} for *L. macrochirus* was determined to be 57 mg a.s./L (95% confidence interval 45-73 mg trinexapac-ethyl/L). The NOEC (Common carp, 96h) = 32 mg trinexapac-ethyl/L. The results are based on the nominal test substance concentrations. Analytical results: The levels of trinexapac-ethyl were measured at 0 and 96 hours. The mean measured values ranged from 92% to 110% of the nominal values. Validity criteria were met in accordance with OECD guideline 203 (1984): Control mortality was 0% and dissolved oxygen was retained above 60% saturation.

The sensitivity of *Ictalurus punctatus* (channel catfish) to trinexapac-ethyl was determined in a GLP-compliant flow-through test performed to US EPA/FIFRA Guideline 72-1 (Anonymous 1991a (CA8.2.1/04)). The 96-hour LC_{50} for *L. macrochirus* was determined to be 35 mg a.s./L (95% confidence interval 31-45 mg trinexapac-ethyl/L). The NOEC (*Ictalurus punctatus*, 96h) = 20 mg trinexapac-ethyl/L. The results are based on the mean measured test substance concentrations.

The lowest LC50 result for fish, the 96-h LC50 of 35 mg a.s./L in channel catfish (Anonymous, 1991a).

Therefore, a full summary for this study is reported below:

Report: CA8.2.1/04	**Anonymous, (1991a)** **Acute toxicity to channel catfish (*Ictalurus punctatus*) under flow-through conditions**

Report No: Confidential

Guidelines: US EPA/FIFRA Guideline 72-1

GLP: Yes

Previous evaluation: In DAR (October, 2003);

Materials and Methods:

***Test substance*:** trinexapac-ethyl (purity 92.2%)

Batch: FL 891393

***Test species*:** channel catfish (*Ictalurus punctatus*)

***Number of organisms, weight, length*:** 20 fish/treatment (duplicate aquaria containing 10 fish each per treatment and control). Mean standard length of 54 (46–62) mm; Mean standard weight of 1.24 (0.72–1.81) g.

***Type of test*:** 96h flow-through acute toxicity test

***Applied and measured concentrations*:** Nominal test concentrations: 13, 21, 32, 49, 75 mg/L and two controls (dilution water and solvent). Measured concentrations were 92-101% of the nominal values.

***Test conditions*:**

Temperature: 22 -23 °C

pH: 6.5 – 6.8

Oxygen content: 75 – 86% of saturation value

Photoperiod: 16:8 hours light:dark

Water hardness: 35 – 36 mg/L $CaCO_3$

***Analytical methods*:** HPLC

Findings:

Analytical results: The levels of CGA163935 were measured at 0 and 96 hours. The mean measured values ranged from 92% to 101% of the nominal values.

Mortality: No control mortality, full mortality at 45 and 75 mg/L. LC_{50} was estimated as 35 mg/L.

Mortalities at different treatment levels following 96 h of exposure

Concentration mean measured (mg trinexapac-ethyl/L)	**0**	**12**	**20**	**31**	**45**	**76**

Mortality (%)	0	0	0	15	100	100

Behavioural observations: In the second and third highest dosage groups some fish showed erratic swimming, partial loss of equilibrium or swimming at the surface of the test solution.

Comments RMS:

Study is acceptable. Validity criteria were met in accordance with OECD guideline no.203 (1984). Control mortality was 0% and dissolved oxygen was retained above 60% saturation and the tested concentrations were confirmed as within 92-101% nominal throughout the study duration.
LC_{50} (*Ictalurus punctatus*, 96h) = 35 mg /L (95% confidence interval 31-45 mg trinexapac-ethyl/L), based on mean measured concentrations.
NOEC (*Ictalurus punctatus*, 96h) = 20 mg trinexapac-ethyl/L.

The sensitivity of *Cyprinodon variegatus* (sheepshead minnow) to trinexapac-ethyl was determined in a GLP-compliant flow-through test peformed to EPA Guideline 72-3 (Anonymous, 1991b (CA8.2.1/05)). The 96-hour LC_{50} for *Cyprinodon variegatus* was determined to be 180 mg a.s./L (95% confidence interval 160-200 mg trinexapac-ethyl/L)). The NOEC (*Cyprinodon variegatus*, 96h) = <60 mg trinexapac-ethyl/L. The results are based on the mean measured test substance concentrations. The mortality in the control does not exceed 10 % (or one fish if less than 10 are used) at the end of the study (being: 0%). The constant conditions were maintained as far as possible throughout the test. The dissolved oxygen concentration was at least 60% of the air saturation value throughout the test.

The lowest LC_{50} is the 96-hour of 35 mg a.s./L in fresh water species *Ictalurus punctatus* (Anonymous,1991a) (CA8.2.1/04) and this result is considered for classification purposes. However, this endpoint is less sensitive then aquatic plants (LC_{50} – 0.20; see 2.9.2.3.3 below) and therefore the endpoint does not determine the classification for trinexapac-ethyl.

2.9.2.2.2 Acute (short-term) toxicity to aquatic invertebrates

Acute aquatic invertebrates toxicity tests were conducted in one freshwater and two marine species. All are considered to be relevant, reliable and adequate for classification purposes. The 48 h EC_{50} value in *Daphnia magna* is >142.5 mg a.s./L, based on nominal test concentrations. Marine species tested *Crassostrea virginica* and *Mysidopsis bahia* observed 96 h EC_{50}/LC_{50} values, based on mean measured concentrations, of 89 and 6.5 mg a.s./L, respectively.

The sensitivity of *Daphnia magna* to trinexapac-ethyl was determined in a GLP-compliant semi static test performed to US EPA/FIFRA Guideline 72-2 (Smith *et.al.*, 1990). The 48-hour LC_{50} for *Daphnia* exposed to the test substance was determined to be >142.5 mg a.s./L (95% confidence interval not applicable because the LC_{50} value was greater than the highest concentration tested). The NOEC (*Daphnia*, 48h) = 29 mg trinexapac-ethyl/L. The results are based on the nominal test substance concentrations. Analytical results: The levels of CGA163935 were measured in the low, middle and highest exposure solutions on each day of the 48 hour test. The sample detection limit was 0.2 mg/L, and the mean measured values ranged from 102% to1 05% of the nominal values. Validity criteria were met in accordance with OECD guideline no. 202 (2004). There was 5% mortality (immobilization) in the control. The dissolved oxygen range of 8.3 to 9.0 mg/L. The maximum concentration of acetone solvent used was 0.467 ml/L (solvent control and the highest test material exposure concentration) which is less than the 0.5 ml/L limit specified in the study specific protocol. The guidelines limiting the solvent to a maximum of 100 mg/L are been introduced since the study was undertaken. There were no mortalities (or immobilizations) in the dilution water or solvent control, or in the CGA-163935 nominal concentrations of 100 mg/L or less during the 48 hour exposure. It is unlikely that the additional solvent used for the high rate treatments will have impacted the results as the study included two controls one with and one without solvent and no differences were seen between the two controls.

The sensitivity of *Mysidopsis bahia* to trinexapac-ethyl was determined in a GLP-compliant flow-through test performed to EPA Guideline 72-3 (Sousa, 1991). The 96-hour LC_{50} for *M.bahia* exposed to the test substance was determined to be 6.5 mg a.s./L (95% confidence interval 5.8 – 7.5 mg trinexapac-ethyl/L). The NOEC (*M.bahia*, 96h) = < 3.4 mg trinexapac-ethyl/L. The results are based on mean measured test substance concentrations.

The lowest LC50 result for aquatic invertebrates, the 96-h LC50 of 6.5 mg a.s./L in *Mysidopsis bahia*, (Sousa, 1991).

Therefore, a full summary for this study is reported below:

Report:	**Sousa J.V. (1991), Acute toxicity to mysid shrimp (*Mysidopsis bahia*) under flow-through conditions, Report Number 91-1-3603.**

Guidelines: EPA Guideline No. 72-3

GLP: Yes

Executive Summary

The acute toxicity of CGA163935 technical to the saltwater mysid (*Mysidopsis bahia*) was determined under flow-through conditions. This study was run with nominal concentrations of 12, 7.8, 5.1, 3.3, and 2.1 mg a.s./L (12, 8.7, 5.9, 4.1 and 3.4 mg/L mean measured) together with negative and solvent controls.

The 96 hour LC_{50} was 6.5 mg a.s./L based on mean measured concentrations.

Materials

Test material	CGA163935 (Trinexapac-ethyl) Technical
Description:	Dark amber liquid
Lot/Batch #:	FL-891393

Actual content of a.i.: 92.2%

Stability of test compound: Not given

Treatments

Test concentrations: 12, 7.8, 5.1, 3.3, and 2.1 mg a.s./L nominal (12, 8.7, 5.9, 4.1 and 3.4 mg/L mean measured)

Dilution water: Saltwater (0.25 µm filtered seawater)

Vehicle and/or positive control: Dimethylformamide (DMF)

Analysis of test concentrations: Yes at 0 and 96 hours using HPLC analysis

Test organisms

Species: Saltwater mysid (*Mysidopsis bahia*)

Source: Test facility

Acclimatisation period: Not stated

Treatment for disease: None

Life stage of test organism: Juvenile

Feeding: Live brine shrimp (*Artemia* sp.) 2-3 times per day during test

Test design

Test vessels: Glass aquaria (29.25 × 14.5 x 19 cm)

Replication: 2 replicates, 10 mysids per replicate

Exposure regime: Flow-through

Duration: 96 hours

Environmental conditions

Test temperature: 25 °C

pH range: 7.7 to 8.0 measured daily

Dissolved oxygen: 3.1 to 7.3 mg/L measured daily (44 – 106 % Saturation)

	Range of dissolved oxygen concentration (% Saturation)				
Time (hours)	0	24	48	72	96
Control	103-104	102-106	94-103	81-102	58-96
4 lowest rate treatments (2.1, 3.3, 5.1, 7.8 mg/L)	90-93	71-90	61-75	57-68	**44-57**
Highest rate treatment (12 mg/L_	91-96	74-78	61-61	**46-58**	**45-46**

	A drop in dissolved oxygen (DO) level below 60% was first measured at 72 hours for the highest rate treatment, but the mean measured concentration for the other 4 treatments was 59% so the overall the DO levels were still very close to the acceptable limit. Aeration was introduced on day 3 to try to increase DO levels, but despite this at 96 hours the mean measured concentration for all treatments had fallen to an average of 47% (with a range from 44-57%). The experimentalists considered that these deviations from the protocol did not affect the results of the study.
Salinity of dilution water:	31‰
Lighting:	16 hours fluorescent light and 8 hours dark daily. Light intensity ≈ 62 footcandles

Study Design and Methods

Experimental dates: 29 November to 3 December 1990

The test chambers were impartially positioned within a water bath to maintain temperature . Two replicate tanks were prepared for the controls and each test solution. Ten mysids were randomly allocated to each prepared test vessel.

A primary stock of 126 mg a.s./mL was prepared by dissolving 13.6702 g of CGA163935 Technical with DMF to volume in a 100 mL volumetric flask. The test stocks were injected into the diluter mixing chambers where they were mixed with saltwater to achieve the desired test concentrations. The resultant test concentrations were adjusted for purity of the active ingredient in test substance. DMF only was injected into the mixing chamber for the solvent control group.

The concentrations of test material in the test solutions were measured at the beginning, and at 24, 48, 72 and 96 hours using liquid scintillation based on the radiolabelled content.

Observations were made for mortality and clinical symptoms of toxicity at approximately 24, 48, 72 and 96 hours.

Results and Discussion

Mean measured concentrations were used for the calculation and reporting of results, as shown in the table below.

Analytical results

Nominal concentration (mg a.s./L)	Measured concentration at 0 hours (mg/L)	Measured concentration at 96 hours (mg/L)	Mean measured concentration (mg a.s./L)
12	11.65	12	12
7.8	9.85	7.6	8.7
5.1	7.1	4.75	5.9
3.3	4.7	3.5	4.1
2.1	3.6	3.05	3.4

Toxicity symptoms (e.g. lethargy, darkened pigmentation and erratic swimming) appeared in the 3.4 mg/L treatment and above. Mortality was observed from 4.1 mg/L and above. Mortalities were observed in the control, but were low enough for the test to still be considered valid.

Effects of CGA163935 Technical on the survival of saltwater mysids (*Mysidopsis bahia*) following exposure for 96 hours in a flow-through test

Mean measured concentration	Cumulative mortality (%)			
(mg a.s./L)	**24 hour**	**48 hour**	**72 hour**	**96 hour**
Dilution water control	5	5	5	5
Solvent control	0	0	0	0
12	5	70	95	95
8.7	5	50	60	70
5.9	0	15	25	30
4.1	5	10	10	25
3.4	0	0	0	0
LC_{50} (mg a.s./L)	>12	9.1	7.2	6.5
95% confidence limits	n.d.	7.8 - 11	6.5 – 8.3	5.8 – 7.5
NOEC (mg/L)	< 3.4			

n.d. – not determined

Conclusions:

The 96 hour LC_{50} for trinexapac-ethyl to the saltwater mysid (*Mysidopsis bahia*) was calculated to be 6.5 mg a.s./L, based on mean measured concentrations.

Comments:

The study was conducted to the US EPA test guideline.

The mortality in the control group was below 10% (being: 5 % in the control and 0 % in the solvent control).

At 72 hours of the definitive study, the total dissolved oxygen dropped below 60 % till 44 %. Dissolved oxygen

levels never fell below 44% of saturation.
During the study in one replicate of the control established a temperature range of 23-24°C and 8 litter all-glass aquaria were used.
The RMS is of the opinion that the deviations did not affect the results. Hence, the results of the study are acceptable and should be used in the risk assessment.

The sensitivity of *Crassostrea virginica* to trinexapac-ethyl was determined in a GLP-compliant flow-through test peformed to EPA Guideline 72-3 (Dionne, 1991). The 96-hour LC_{50} for *C.virginica* exposed to the test substance was determined to be 89 mg a.s./L (95% confidence interval 50 – 180 mg trinexapac-ethyl/L) based on shell deposition. The NOEC (*C.virginica*, 96h) = < 8.4 mg trinexapac-ethyl/L. The results are based on mean measured test substance concentrations. Mean measured concentrations, calculated from the average of all samples, ranged from 76 to 110% of nominal concentrations. Mean measured concentrations were used for the reporting of the results. Validity criteria were met: The mortality in the control group was below 10% (being: 0 % in the control and 0 % in the solvent control). The dissolved oxygen concentration should be at least 60 % (was > 60 %). Significant differences ($p \leq 0.05$) were between growth of dilution water and solvent control oysters, thus the solvent control was used when comparing treated and control data. The concentration of the test substance was maintained over the test period. The environmental conditions (temperature, dissolved oxygen, salinity and pH were measured at the beginning and at the end of the test.

The lowest LC_{50} is the 96-hour of 6.5 mg a.s./L in saltwater species *Mysidopsis bahia* (Sousa,1991) and this result is considered for classification purposes. However, this endpoint is less sensitive then aquatic plants (LC_{50} – 0.20; see 2.9.2.3.3 below) and therefore the endpoint does not determine the classification for trinexapac-ethyl.

2.9.2.2.3 Acute (short-term) toxicity to algae or aquatic plants

Please refer to Section 2.9.2.3.3 "chronic toxicity to algae or aquatic plants" where both acute (short-term) and chronic toxicity to algae and aquatic plants are discussed.

2.9.2.2.4 Acute (short-term) toxicity to other aquatic organisms

No toxicity test with the sediment dwelling midge *Chironomus* spp. was deemed necessary for trinexapac-ethyl, trinexapac (CGA179500) or other metabolites. No new data are provided. (For more information see volume 3-B.9 (AS)). Due to the short residence time of trinexapac-ethyl in the aquatic system and its moderate toxicity to *D. magna* ($NOEC_{21\ days}$ 2.4 mg/L) no toxicity test with the sediment dwelling midge *Chironomus* spp. was deemed to be necessary for the parent compound. Also due to considerations of the mode of action for trinexapac-ethyl, a study has been conducted in artificial sediment with the rooted macrophyte *Myriophyllum spicatum*. Measured sediment concentrations of both trinexapac-ethyl and trinexapac (CGA179500) were all at low levels at test termination and CGA300405 sediment concentrations were < LOQ at all sampling occasions.

2.9.2.3 Long-term aquatic hazard

Table 73: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Key or Supportive study	Remarks	Reference
EPA guideline No. 72-4	**Fathead minnow (*Pimephales promelas*)**	**trinexapac-ethyl (purity 92.2%)**	**35d-NOEC 0.41 mg a.s./L (mm)**	**Key**	**0.41 mg a.s./L**	**Anonymous, (1991) CA8.2.2.1/01 CGA163935 /0189**
US EPA/FIFRA Guideline 72-4	*Daphnia magna*	trinexapac-ethyl (purity 92.2%)	21d-NOEC 11 mg a.s./L (mm)			Putt (1991) CGA163935 / 161
US EPA/FIFRA Guideline 72-4	***Daphnia magna***	**trinexapac-ethyl (93.8%)**	**21d-NOEC 2.4 mg a.s./L mm)**	**Key**	**2.4 mg a.s./L**	**Putt (1994) CGA163935/0370**
OECD 201	Green alga *(Pseudokirchneriella subcapitata)*	trinexapac-ethyl (96.8%)	72 h-E_rC_{50} 60 mg a.s./L (mm)			Maetzler (2001) CGA163935/0695
OECD 201 O.J. L383A, Part C.3: Algal inhibition test (1992) US EPA Guideline OPPTS 850.5400 Algal Toxicity, Tiers I and II, (1996)	**Green alga *(Pseudokirchneriella subcapitata)***	**trinexapac-ethyl (95.8%)**	**96h-E_rC_{50} 24.5 mg a.s./L NOEC 8 mg a.s./L (nom)**	**Key**	**8 mg a.s./L**	**Cartee *et al.* (2009) CGA163935_10480**
OECD Guideline 201 (2006) EU Commission Directive 92/69/EEC, C.3 (1992)	Green alga *(Pseudokirchneriella subcapitata)*	trinexapac-ethyl (97.4%)	72 h-E_rC_{50} 61 mg a.s./L (nom)			Bätscher (2008) Adama study no. B93014 CGA163935_10659
OECD Guideline 201 (2006)	Green alga *(Pseudokirchneriella subcapitata)*	trinexapac-ethyl (98.4%)	72h-E_rC_{50} 41.6 mg a.s./L (nom)			Scheerbaum (2008) Cheminova Report Doc. No.: 77 TPE CGA163935_106

						69
OECD Guideline 201 (2006)	Blue-green alga *(Anabaena flos-aquae)*	trinexapac-ethyl (97.4%)	72h-E_rC_{50} >100 mg a.s./L NOEC 46 mg a.s./L (nom)			Liedtka (2010) Adama study no. B92867 CGA163935_10662
OECD Guideline 201 (2006)	Blue-green alga *(Anabaena flos-aquae)*	trinexapac-ethyl (98.4%)	72 h-E_rC_{50} 295 mg a.s./L (nom)			Scheerbaum (2008b) Cheminova Report Doc. No.: 76 TPE CGA163935_10668
FIFRA Guideline 122-2 and 123-2, ASTM E 1415-91 and OECD (draft December 1999)	*Lemna gibba*	trinexapac-ethyl (96.8%)	7 d- E_rC_{50} 27.4 mg a.s./L (nom)			Grade (2001) CGA163935/708
OECD Guideline 221 (2006)	*Lemna gibba*	trinexapac-ethyl (97.4%)	7 d-E_rC_{50} 65 mg a.s./L (mm)			Bätscher (2008b) Adama study no. B92891 CGA163935_10660
OECD Guideline 221 (2006)	*Lemna gibba*	trinexapac-ethyl (98.4%)	7 d-E_rC_{50} 36.1 mg a.s./L (nom)			Scheerbaum (2008c) Cheminova Report Doc. No.: 78 TPE CGA163935_10671
OECD Guidelines 239 (2014)	***Myriophyllum spicatum***	**trinexapac-ethyl (95.4%)**	**14 d** **shoot length** **E_rC_{50} 1.2 mg a.s./L** **Shoot wet wt** **E_rC_{50} 1.4 mg a.s./L** **shoot dry wt** **E_rC_{50} > 8.8 mg a.s./L** **NOEC <0.025**	**Key**	**<0.025 mg/L**	**Kirkwood (2015) CGA163935_10672**

			mg/L (mm)			

2.9.2.3.1 Chronic toxicity to fish

A fish early life-stage toxicity study with the Fathead minnow (*Pimephales promelas*) was conducted according to EPA/FIFRA guideline No. 72-4 and GLP (Anonymous, 1991 (CA8.2.2.1/01)). This study is considered to be relevant and reliable and adequate for classification purposes. The chronic NOEC value in a flow-through test was 0.41 mg a.s/L (mean measured concentration) based on development and growth parameters. (The edpoints were egg hatchability, survival and growth (length and dry weight)). However, this endpoint is less sensitive then aquatic plants endpoint (NOEC < 0.025; see 2.9.2.3.3 below) and therefore the endpoint does not determine the classification for trinexapac-ethyl.

However, for transparency a full summary for this study is reported below:

Report: CA8.2.2.1/01	**Anonymous. (1991)** **CGA-163935 – toxicity to fathead minnow (*Pimephales promelas*) embryos and larvae**

Report No: Confidential

Guidelines: US EPA/FIFRA Guideline 72-4

GLP: Yes

Previous evaluation: In DAR (October 2003); relevant for renewal application

Materials and Methods:

Test substance: trinexapac-ethyl (purity 92.2%) in dimethylformamide (0.027 mL/L which equals 25 mg/L)

Batch No: FL 891393

Test species: Fathead minnow (*Pimephales promelas*)

Number of organisms: 60 per treatment

Type of test: 35-days flow-through fish early-live stage toxicity test

Mean measured concentrations: two untreated control (dilution water and solvent), 3.0, 1.5, 0.80, 0.4, and 0.20 mg/L

Test conditions*:*

Temperature: 23.0 – 27.0 °C

pH: 6.8 -7.1

Oxygen content: 6.7 -8.0 mg/L

Flow rate: at least 15 volume turnovers in the test aquaria per 24h

Analytical methods: HPLC

Study design:

Fourteen test aquaria with 60 embryos in each were set up. The definitive embryo exposure was initited within 24 hours after egg fertilization and continued through 35 days. The effects on embryo survival at hatch and on survival and growth (wet weight and total length) of larvae at test termination were measured and used to estimate the MATC.

Observations were made on survival of organisms at hatch and on the survival and growth (wet weight and total length) of larvae after 30 days of post-hatch exposure. Actual concentrations of trinexapac were measured at 0 and 96 h by HPLC after dilution with a mixture of acetonitrile and water (spike recovery: 101±5%). Water quality parameters were within accepted range. Measured concentrations were 100 - 108 % of the nominal values.

Findings:

Analytical results: During the 35 day study weekly analyses established that the diluter system functioned properly and the mean measured values averaged 104% of the nominal values with a range from 100% to 108% of the nominal values.

Survival of the fathead minnows at the end of the pre-hatch period (five days after starting eggs incubation) was comparable with the pooled controls (78 – 84% versus 79%, respectively). At the end of the post-hatch period, the number of surviving larvae at the top-dose was significantly lower than in the pooled controls (91% versus 98%, respectively). At the other dosages there were no significant differences in larvae survival. Only the two lowest dosages showed a comparable increase in total length and weight as the pooled controls. Therefore growth inhibition occurred as from dosages of 0.80 mg/L (actual) and higher, thus being the most susceptible end-point next to mortality. A few larvae in control and trinexapac-treatments showed darkened pigmentation, small body size, and spinal deformity. MATC was reported as >0.41 and <0.81 mg/L, geometric mean MATC 0.57 mg/L.

Trinexapac-ethyl: Fathead minnow early-life stage toxicity test: summary of concentration effect data.

Concentration (mg/L) [a]	Embryos hatched (%) [b]	30-day larval survival (%) [b]	Mean wet weight (mg)	Mean length (mm)
3	78	91*	173	27
1.5	82	96	213	28.9
0.8	84	95	273	31.4
0.41	78	99	311	32.9
0.2	82	96	308	32.7
pooled control	79	98	319	33.2

Note: [a]*Mean measured concentration and standard deviation*
[b]*Based on 60embryos per replicate*
[c]*Based on number of embryos that hatched*
[*]*Statistically less than pooled controls* ($p \leq 0.05$)

Comments:

Validity criteria were met in accordance with OECD guideline no. 210 (2013).

Dissolved oxygen was retained above 60% saturation. The water temperature range for specified species = 25±2°C. Measured concentrations were 100 - 108 % of the nominal values during the test. The test concentration was sufficiently maintained during the test period. Achieved control hatching success = 79% and post-hatch survival = 98% (should be greater than or equal to the limits defined for *P.promelas* = 70 and 75% accordingly). There were some noted deviation as the temperature ranged from 23 to 27°C (should be = 25±1.5°C). However, it can be considered that this deviation did not affect the results of this study. The study is acceptable.

NOEC (*Pimephales promelas*, 32 day) = 0.41 mg trinexapac-ethyl/L (based on mean measured concentrations).

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

Two studies with freshwater *Daphnia magna* are available. Both are considered to be relevant, reliable and adequate for classification purposes. The toxicity of the test item to the chronic survival and reproduction of *Daphnia* was determined in a GLP-compliant tests performed to US EPA/FIFRA Guideline 72-4 (Putt, 1991) amd (Putt, 1994).

The chronic 21 d NOEC values in a flow-through tests were 11 mg a.s/L and 2.4 mg a.s/L (mean measured concentration) based on mortality, reproduction and growth parameters. However, these endpoints are less sensitive then aquatic plants (NOEC < 0.025; see 2.9.2.3.3 below) and therefore the endpoints do not determine the classification for trinexapac-ethyl.

However, for transparency a full summary for this study is reported below:

Report:	**Putt A.E. (1994)** **CGA-163935 technical-the chronic toxicity to daphnids (*Daphnia magna*) under flow-through conditions.**

Report No: 93-6-4810

Guidelines: US EPA/FIFRA Guideline 72-4

GLP: Yes

Previous evaluation: In DAR (October, 2003);

Materials and Methods:

Test substance: trinexapac-ethyl (93.8% a.s.), Batch No. FL-911999

Test species: Daphnia magna

Number of organisms, age: 10 daphnids (≤ 24 hours old) per vessel, four vessels per treatment.

Type of test: 21d flow-through chronic toxicity test

Applied and measured concentrations:

Five nominal test concentrations: 3.1, 6.3, 13, 25 and 50 mg trinexapac-ethyl/L

Mean measured concentrations: 2.4, 5.1, 10, 21 and 43 mg trinexapac-ethyl /L (84 - 95% of nominal)

Test conditions*:*

Temperature: 19 - 21°C

pH: 7.9 – 8.3

Oxygen content: 6.8 – 8.9 mg dissolved oxygen/mL

Photoperiod: 16:8 hours light:dark

Water hardness: 160 – 180 mg/L $CaCO_3$

Test parameters: survival of first generation daphnids, the dry weight of the first generation daphnids at the conclusion of the test, the time to first brood and production of young by the first generation daphnids. The number of surviving adult daphnids. At test termination surviving adults were dried and weighed.

Measurements of pH, temperature and dissolved oxygen concentrations were periodically measured during the test. Temperature was also recorded continuously.

Statistics: All statistical conclusions were made at the 95% level of certainty except in the case of the Chi-Square Goodness of Fit Test and the Bartlett`s Test, in which the 99% level of certainty was applied. The theoretical threshold concentration expected to produce no deleterious effects at the 95% level of certainty was estimated as the maximum acceptable toxicant concentration (MATC). Based on this data, the MATC of CGA-163935 to daphnia magna was established to be > 2.4 and < 5.1 mg a.i./L (geometric mean MATC-3.5 mg a.i./L).

Analytical methods: Actual concentrations were measured at 0, 7, 14 and 21 days by HPLC (recovery 99.7 ± 5%).

Findings:

Analytical results: During the 21 day study concentrations of CGA-163935in replicate exposure solutions were measured at 0, 7, 14 and 21 days and the mean measured values were found to be 43, 21, 10 5.1 and 2.4 mg/L. These values were used to determine the endpoints.

Water quality parameters were within accepted range. The NOEC in the heading table was based on mortality (young and adult), adult growth, adult length and reproduction. The most sensitive endpoint was the length of the surviving parental daphnids. Mean measured concentrations were 77 - 86% of nominal. The adult mortality in the treatment groups was comparable with the adult mortality in the control (max. 42%: statistically insignificant). First brood after 8 days (not different from the control).

The results of visual inspection of clinical effects were not reported. Although this inspection was not specifically required from the test protocol, it is not clear whether visual inspection did not occur or did occur, though without reporting the results. The result actual 21-d NOEC 2.4 mg/L is used for risk assessment

Survival, reproduction and weight data (mean values for each tested concentration) from the chronic toxicity test with daphnids, *Daphnia magna*, and trinexapac-ethyl/L

Mean measured concentration (mg/L)	Percent survival at 21 days	Cumulative number of offspring produced per female	Mean total body lengths (mm) at 21 days	Mean dry weights (mg) at 21 days
(control)	100	217	5.5	1.48
2.4	95	224	5.5	1.36
5.1	68	187	5.3	1.28
10	58[a]	161*	5.2*	1.18
21	83[a]	175*	5.2*	1.44
43	95	144*	5.4*	1.57

Note: a. not significantly different as compared to the control and not considered toxicant-related based on the absence of similar reductions at higher treatment levels.
** significantly different ($p \leq 0.05$) as compared to the control data*

Conclusions:

Endpoints for 21d mortality (young and adult), adult growth, adult length and reproduction (based on mean measured concentrations):

NOEC (*Dapnia magna*, 21 day) = 2.4 mg trinexapac-ethyl/L

Comments:

The study was conducted to the US EPA test guideline.
The control survival and reproduction (100% and 217 offspring per female) met the minimum standard criteria: (i.e., no more than 20 % of the control organisms are immobilized, stressed or diseased, > 60 offspring per female). The concentration of the test substance was maintained over the test period. The environmental conditions (temperature, dissolved oxygen, salinity and pH) were measured during the test.

The RMS is of the opinion that the results of the study are acceptable and should be used in the risk assessment

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

Six algal studies (four with Green algae and two with Blue-green algae (cyanobacterium)) are available or this endpoint. All are considered to be relevant, reliable and adequate for classification purposes.

The toxicity of trinexapac-ethyl to the green algae *Pseudokirchneriella subcapitata* was tested in a GLP-compliant static test performed to OECD 201 (Maetzler, 2001). The 72-hour E_bC_{50} value was 27 mg a.s./L based on biomass and the 72-hour E_rC_{50} value was 60 mg a.s./L based on growth rate. The 72 hour NOEC was 9.4 mg trinexapac-ethyl/L for biomass, growth rate. The results are based on the mean measured test substance concentrations. Actual concentrations of trinexapac-ethyl were measured at 0 and 96 hours. Mean measured concentrations were in range of 82.2%- 95.5% of nominal concentration over the whole test duration. Validity criteria were met: The mean cell density in the control increased by a factor ≥ 16 (measured: cell density increased by a factor 81). The mean coefficient of variation for section-by-section specific growth rates in the control cultures does not exceed 35% (measured: 11%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% (measured: 2.2%).

The toxicity of trinexapac-ethyl to the green algae *Pseudokirchneriella subcapitata* was tested in a GLP-compliant static test performed to OECD 201 and US EPA Guideline (Cartee, 2009). The 96-hour both E_bC_{50} and E_yC_{50} values were 14.3 mg a.s./L based on biomass and yield and the 96-hour E_rC_{50} value was 24.5 mg a.s./L based on growth rate. The 96 hour NOEC was 8 mg trinexapac-ethyl/L for biomass, growth rate and yield. The results are based on the nominal test substance concentrations.

Report:	**Cartee, T.L., Kendall, T.Z., and H.O. Krueger. 2009.** **Trinexapac-ethyl - A 96-Hour Toxicity Test with the Freshwater Alga (*Pseudokirchneriella subcapitata*)**

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006)

Official Journal of the European Communities, Dir 92/69/EEC, O.J. L383A, Part C.3: Algal inhibition test (1992)

US EPA Ecological Effects Test Guidelines, OPPTS 850.5400: Algal Toxicity, Tiers I and II, (1996)

GLP: Yes

Executive Summary

The toxicity of trinexapac-ethyl to the green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to nominal concentrations of 2.0, 4.0, 8.0, 16, and 32 mg a.s./L alongside a culture medium control. Based on nominal concentrations, the 72-hour E_rC_{50} was 24.9 mg a.s./L, the E_yC_{50} was 14.5 mg a.s./L and the E_bC_{50} was 14.5 mg a.s./L. The 96-hour E_rC_{50} was 24.5 mg a.s./L, the E_yC_{50} was 14.3 mg a.s./L and the E_bC_{50} was 14.3 mg a.s./L.

Materials

Test Material	Trinexapac-ethyl technical
Batch/Lot #:	573928 (SMO8E551)
Purity:	95.8 (wt/wt)

Description:	Yellow to reddish brown solid (as indicated on certificate of analysis)
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	August 2012

Treatments

Test concentrations:	Culture medium control and nominal concentrations of 2.0, 4.0, 8.0, 16 and 32 mg a.s./L
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, analysis of trinexapac-ethyl at 0 and 96 hours

Test organism

Species:	*Pseudokirchneriella subcapitata*
Source:	Continuous laboratory cultures, originally obtained from the University of Toronto Culture Collection

Test design

Test vessels:	250 mL glass Erlenmeyer flasks containing 100 mL of media plugged with foam stoppers
Test medium:	AAP algal medium
Replication:	Six vessels for the control and three vessels for each test concentration
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	96 hours

Environmental conditions

Test temperature:	22.8 – 24.6°C	
pH:	test start:	6.8 to 7.6
	test end:	7.1 to 8.5
Lighting:	Continuous illumination at 4040 to 4620 Lux	

Study Design and Methods

Experimental dates: September 28 to October 2, 2009.

A stock solution with a nominal concentration of 32 mg a.s./L was prepared by dissolving 33.4 mg of the test item completely in 1000 mL of test medium. Appropriate volumes of the stock solution were diluted to give the test concentration series. The control consisted of culture medium only.

An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 10,000 algal cells per mL of test medium. Test solutions were constantly shaken and were held in a temperature controlled incubator under continuous illumination.

Small volumes of all test concentrations and controls were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in these samples were determined by counting with an electronic particle counter. In addition, after 96 hours exposure, samples were taken from the control and from all test concentrations. The shape of the algal cells was examined microscopically in these samples.

The pH was measured at the start and at the end of the test. The water temperature was measured daily in a flask incubated under the same conditions as the test flasks.

The test concentrations were verified by chemical analysis of trinexapac-ethyl at 0 and 96 hours, using high performance liquid chromatography.

Results and Discussion

At the start of the test, the measured concentrations were in the range 98 to 100% of the nominal values and at the end of the test were in the range 93 to 97% (see table below). The limit of quantification in this study was 1.0 mg a.s/L. Nominal concentrations were used for the calculation and reporting of results.

Analytical results

Nominal concentration (mg a.s/L)	% of nominal measured at 0 hours	% of nominal measured at 96 hours	Mean measured concentration (mg a.s./L)
Control	--	--	--
2.0	99.8	93.5	1.9
4.0	98.2	93.1	3.8
8.0	99.6	95.3	7.8
16	98.6	96.6	16
32	97.8	97.0	31

The algal cell densities were measured at 24, 48, 72 and 96 hours and the mean biomass, growth rate and yield calculated. The 72-hour and 96-hour E_bC_{50}, E_yC_{50} and E_rC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) were calculated using non-linear regression analysis. For determination of the NOEC (No Observed Effect Concentration) values, a Dunnett's test was used to identify significant differences in the calculated mean biomass, growth rate and yield of test item treatments compared to the control.

There were no abnormalities, observed microscopically, in the control or in any test concentration at 96 hours.

Growth rates

The growth rate 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Mean values at each concentration of trinexapac-ethyl for the growth rate at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Nominal concentration (mg a.s./L)	Mean growth rate (1/day) 0 – 72 hrs	Percentage inhibition	Mean growth rate (1/day) 0 – 96 hrs	Percentage inhibition
Negative Control	0.0664	--	0.0627	--
2.0	0.0681	-2.6	0.0625	0.3
4.0	0.0683	-2.8	0.0626	0.2
8.0	0.0677	-1.8	0.0627	0
16	0.0544	18	0.0522	17
32	0.0209	69	0.0172	73
E_rC_{50} mg a.s./L (95% confidence limits)	24.9 (24.1 – 25.7)		24.5 (24.1 – 24.9)	
NOEC	8.0 mg a.s./L		8.0 mg a.s./L	

(- value) = Increase in growth compared to the control

Yield

The yield 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Mean values at each concentration of trinexapac-ethyl for the yield at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Nominal concentration (mg a.s./L)	Mean yield (cells/mL) 0 – 72 hrs	Percentage inhibition	Mean yield (cells/mL) 0 – 96 hrs	Percentage inhibition
Negative Control	1,203,287	--	4,125,090	--
2.0	1,341,679	-12	4,035,482	2.2
4.0	1,381,875	-15	4,105,120	0.5
8.0	1,294,349	-7.6	4,116,463	0.2
16	493,776	59	1,490,441	64
32	34,933	97	42,181	99
E_yC_{50} mg a.s./L (95% confidence limits)	14.5 (12.7 – 16.5)		14.3 (13.5 – 15.1)	
NOEC	8.0 mg a.s./L		8.0 mg a.s./L	

(- value) = Increase in growth compared to the control

Cell density

The cell density for 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Mean values at each concentration of trinexapac-ethyl for cell density at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Nominal concentration (mg a.s./L)	Mean cell density (cells/mL) 0 – 72 hrs	Percentage inhibition	Mean cell density (cells/mL) 0 – 96 hrs	Percentage inhibition
Negative Control	1,213,287	-	4,135,090	-
2.0	1,351,679	-11	4,045,482	2.2
4.0	1,391,875	-15	4,115,120	0.5
8.0	1,304,349	-7.5	4,126,463	0.2
16	503,776	58	1,500,441	64
32	44,933	96	52,181	99
E_bC_{50} mg a.s./L (95% confidence limits)	14.5 (12.7 – 16.6)		14.3 (13.4 – 15.1)	
NOEC	8.0 mg a.s./L		8.0 mg a.s./L	

(-value) = Increase in growth compared to the control

Conclusions:

Based on nominal concentrations, the 72-hour E_rC_{50} for trinexapac-ethyl to *Pseudokirchneriella subcapitata* was 24.9 mg a.s./L, the E_yC_{50} was 14.5 mg a.s./L and the E_bC_{50} was 14.5 mg a.s./L. The 96-hour E_rC_{50} was 24.5 mg a.s./L, the E_yC_{50} was 14.3 mg a.s./L and the E_bC_{50} was 14.3 mg a.s./L.

Comments:

The validity criteria of OECD Guideline 201 are met:

- The mean cell density in the control increased by a factor ≥ 16 (measured: cell density increased by a factor 121).
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures does not exceed 35% (measured: 12.07%).
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% (measured: 1.25%).
- pH in the control did not increase more than 1.5 units during the study

Consequently, the study is considered acceptable for use in risk assessment. Endpoints are based on nominal concentrations.

The toxicity of trinexapac-ethyl to the green algae Pseudokirchneriella *subcapitata* was tested in a GLP-compliant static test performed to OECD 201 (Bätscher, 2008). The 72-hour E_yC_{50} value was 20 mg a.s./L based on yield and the 72-hour E_rC_{50} value was 61 mg a.s./L based on growth rate. The 72 hour NOEC was 10 mg trinexapac-ethyl/L for growth rate and yield. The measured concentrations of the test substance in the test media of the test concentrations of 10 to 100 mg/L were between 92 and 101% of the nominal values at the start and the end of the test. Therefore, the biological results were related to the nominal concentrations of the test substance.

The validity criteria of OECD Guideline 201 were met: The mean cell density in the control increased by a factor ≥ 16 (measured: cell density increased by a factor 187). The mean coefficient of variation for section-by-section specific growth rates in the control cultures does not exceed 35% (measured: 7%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% (measured: 2.4%). pH in the control did not increase more than 1.5 units during the study.

The toxicity of trinexapac-ethyl to the green algae *Pseudokirchneriella subcapitata* was tested in a GLP-compliant static test performed to OECD 201 (Scheerbaum, 2008). The 72-hour E_yC_{50} value was 22.8 mg a.s./L based on yield and the 72-hour E_rC_{50} value was 41.6 mg a.s./L based on growth rate. The 72 hour NOEC was 10 mg trinexapac-ethyl/L for growth rate and yield. The results are based on the nominal test substance concentrations. The test concentrations were verified by chemical analysis at 0 and 72 hours. At the start of the test, the measured concentrations were in the range 100 to 103% of the nominal values and at the end of the test were in the range 97 to 99%. Nominal concentrations were used for the calculation and reporting of results.

The validity criteria of OECD Guideline 201 were met: The mean cell density in the control increased by a factor ≥ 16 (measured: cell density increased by a factor 114). The mean coefficient of variation for section-by-section specific growth rates in the control cultures does not exceed 35% (measured: 18.60%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% (measured: 2.18%). pH in the control did not increase more than 1.5 units during the study.

The toxicity of trinexapac-ethyl to the blue- green algae *Anabaena flos-aquae* was tested in a GLP-compliant static test performed to OECD 201 (Liedtke, 2010). The 72-hour E_bC_{50} value was >100 mg a.s./L based on biomass and the 72-hour E_rC_{50} value was >100 mg a.s./L based on growth rate. The 72 hour NOEC was 46 mg trinexapac-ethyl/L for biomass and growth rate. The results are based on the nominal test substance concentrations. The test concentrations were verified by chemical analysis at 0 and 72 hours. At the start of the test, the measured concentrations were in the range 100 to 101% of the nominal values and at the end of the test were in the range 93 to 96%. Nominal concentrations were used for the calculation and reporting of results.

The validity criteria of OECD Guideline 201 were met: The mean cell density in the control increased by a factor ≥ 16 (measured: cell density increased by a factor 31). The mean coefficient of variation for section-by-section specific growth rates in the control cultures does not exceed 35% (measured: 24%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 10% (measured: 1.3%). pH in the control did not increase more than 1.5 units during the study.

The toxicity of trinexapac-ethyl to the blue- green algae *Anabaena flos-aquae* was tested in a GLP-compliant static test performed to OECD 201 (Scheerbaum, 2008). The 72-hour E_yC_{50} value was 295 mg a.s./L based on biomass and the 72-hour E_rC_{50} value was 214 mg a.s./L based on growth rate. The 72 hour NOEC was 100 mg trinexapac-ethyl/L for biomass, growth rate and yield. The results are based on the nominal test substance concentrations. The test concentrations were verified by chemical analysis at 0 and 72 hours. At the start of the test, the measured concentrations were in the range 95 to 99% of the nominal values and at the end of the test were in the range 97 to 98%. Nominal concentrations were used for the calculation and reporting of results.

The validity criteria of OECD Guideline 201 were met: The mean cell density in the control increased by a factor ≥ 16 (measured: cell density increased by a factor 62). The mean coefficient of variation for section-by-section specific growth rates in the control cultures does not exceed 35% (measured: 8.38%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 10% (measured: 1.3%). pH in the control did not increase more than 1.5 units during the study.

Four aquatic plants studies are available and these are considered to be relevant, reliable and adequate for classification purposes.

The toxicity of trinexapac-ethyl to duckweed *Lemna gibba* was tested in a GLP-compliant static test performed to FIFRA Guideline 122-2, ASTM E 1415-91 and OECD draft 1999 (Grade, 2001). The 7-day E_bC_{50} value was 8.8 mg a.s./L and the 7-day E_rC_{50} value was 27.4 mg a.s./L based on frond number and dry weight. The 7-day NOEC was 2.3 mg trinexapac-ethyl/L for biomass and growth rate and NOEC (weight) was 7.05 mg trinexapac-ethyl/L. The test concentrations were measured at 0 and 7 days. These measured concentrations were within 89 – 100% of nominal at the start and 67 – 100% at the end. The average mean measured concentrations were used to calculate the endpoints. The results are based on actual mean test substance concentrations.

The validity criteria stated in draft OECD guideline are in line with the current valid OECD test guideline 221 (2006). The doubling time of the frond number in the control was less than 2 days corresponding to an approximately 13-fold increase in biomass in 7 days.

The toxicity of trinexapac-ethyl to duckweed *Lemna gibba* was tested in a GLP-compliant static test performed to OECD 201 (Bätscher, 2008). The 7-day E_yC_{50} value was 11.1 mg a.s./L and the 7-day E_rC_{50} value was 65 mg a.s./L based on frond number and dry weight. The 7-day NOEC was 0.95 mg trinexapac-ethyl/L for growth rate and yield. The concentrations of trinexapac-ethyl were measured in two of the quadruplicate test medium samples of the nominal test concentrations of 1.0 to 100 mg/L from all sampling times. The samples from the lowest nominal test concentrations of 0.10 and 0.32 mg/L were not analysed as the concentrations were below the 7-day NOEC. The average recoveries found in the unaged treatment samples ranged from 92% to 104% (day 0), from 88% to 102% (day 3) and from 94% to 110% (day 5) of the nominal concentrations. The average recoveries found in the aged treatment samples ranged from 83% to 86% (day 3), from 78% to 91% (day 5) and from 79% to 90% (day 7) of the nominal concentrations. Since part of the recoveries for the aged treatment samples were < 80%, the biological results were based on mean measured concentrations of the test substance.

The validity criteria of OECD Guideline 221 were met: increase of frond number in the control was > 7-fold (measured: 15-fold), the doubling time was less than 2.5 days (measured: 1.8 days). Consequently, the study is acceptable for use in risk assessment.

The toxicity of trinexapac-ethyl to duckweed *Lemna gibba* was tested in a GLP-compliant static test performed to OECD 201 (Scheerbaum, 2008). The 7-day E_yC_{50} value was 5.57 mg a.s./L and the 7-day E_rC_{50} value was 36.1 mg a.s./L based on frond number and dry weight. The 7-day NOEC was 1.0 mg trinexapac-ethyl/L for growth rate and yield. At the start of the test, the measured concentrations of Trinexapac-ethyl were in the range 98 to 101% of the nominal values and at the end of the test were in the range 82 to 93%. The limit of quantification in this study was 0.06 mg test item/L. Nominal concentrations were used for the calculation and reporting of results.

The validity criteria of OECD Guideline 221 were met: increase of frond number in the control was > 7-fold (measured: 18-fold), the doubling time was less than 2.5 days (measured: 1.69 days). Consequently, the study is acceptable for use in risk assessment.

The toxicity of trinexapac-ethyl to *Myriophyllum spicatum* was tested in a GLP-compliant static test performed to OECD 239 (2014) (Kirkwood, 2015). The 14-day E_yC_{50} value was 0.60 mg a.s./L and the 14-day E_rC_{50} value was 1.2 mg a.s./L based on shoot length. The 14-day E_yC_{50} value was 0.20 mg a.s./L and the 14-day E_rC_{50} value was 1.4 mg a.s./L based on shoot fresh weight and. The 14-day E_yC_{50} value was 1.9 mg a.s./L and the 14-day E_rC_{50} value was 8.8 mg a.s./L based on shoot dry weight. The 7-day NOEC was < 0.025 mg trinexapac-ethyl/L for growth rate and yield. The results are based on the mean measured test substance concentrations.

Report:	**Kirkwood, A., (2015) Trinexapac-ethyl – Growth Inhibition of the Aquatic Macrophyte *Myriophyllum spicatum* in Water-Sediment System. Report Number 1781.7075**

Guidelines

OECD Guidelines 239: Water-Sediment *Myriophyllum Spicatum* Toxicity Test (2014)

GLP: Yes

Executive Summary

The toxicity of CGA163935 to the aquatic macrophyte *Myriophyllum spicatum* was determined in a 14-day semi- static test. The *Myriophyllum* were exposed to nominal concentrations of 0.030, 0.10, 0.31, 0.98, 3.1 and 10 mg/L (corresponding to 0.025, 0.068, 0.26, 0.78, 2.6 and 8.8 mg/L geometric mean measured) alongside a dilution water control.

For shoot length, the 14-day EC_{50} for yield (EyC_{50}) and growth rate (ErC_{50}) for CGA163935 to *Myriophyllum spicatum* were 0.60 and 1.2 mg ai/L respectively, based on geometric mean measured concentrations. For shoot wet weight, the 14-day EC_{50} for yield (EyC_{50}) and growth rate (ErC_{50}) were 0.20 and 1.4 mg ai/L respectively, based on geometric mean measured concentrations. For shoot dry weight, the 14-day EC_{50} for yield (EyC_{50}) and growth rate (ErC_{50}) were 1.9 and >8.8 mg ai/L respectively, based on geometric mean measured concentrations.

Materials

Test Material	CGA163935 Trinexapac-Ethyl
Batch No.:	SMO4D0962
Purity:	95.4%
Description:	Yellow to red-brown solidified melt
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	31 July 2018
Density:	n/a

Treatments

Test concentrations:	Dilution water control; nominal concentration of 0.030, 0.10, 0.31, 0.98, 3.1 and 10 mg/L (corresponding to 0.025, 0.068, 0.26, 0.78, 2.6 and 8.8 mg/L geometric mean measured)

Solvent:	None
Test item:	1. trinexapac-ethyl - CGA163935; AMS 265/102 2. trinexapac- CGA179500; CGA179500 3. CGA300405; MES 357/1
Analysis of test concentrations:	Yes, analysis on days 0, 1, 3, 7, 10 and 14 for trinexapac-ethyl (parent), trinexapac (degradate) and CGA300405 (degradate), in the test medium. New solution samples, analyzed at days 0 and 7, were removed from the test and control solutions prior to division into the replicate test vessels. Aged solution samples, analyzed at days 1, 3, 7, 10 and 14, were removed from the composited replicate solutions of each test concentration and control.
Analysis of sediment and pore water :	Analysis at days 0, 7 and 14 for the 0.98, 3.1 and 10 mg/L treatment levels and the control
Test organisms	
Species:	*Myriophyllum spicatum*
Source:	In-house cultures originally collected in the Nashua River, Nashua, New Hampshire by the New Hampshire Department of Environmental Services.
Test design	
Test vessels:	4 L glass beakers filled with 3.5 L test solution
Test medium:	Smart & Barko Medium
Biological replication:	Six vessels for the control and four for each test concentration, 3 plants per vessel
Sediment analysis and pore water replication:	3 additional vessels were established for the 0.98, 3.1 and 10 mg/L treatment levels and the control. These contained *Myriophyllum*, and were used only for sediment and pore water analysis, not for biological analysis
Number of plants:	12 plants per test concentration, 18 plants for the control
Exposure regime:	Semi-static; solution renewal on day 7
Duration:	14 days
Environmental conditions	
Temperature:	18 to 22 °C
pH:	7.2 to 9.7
Lighting:	16 hours light, 120 to 150 µE/m^2/s

Study Design and Methods

Experimental dates: 28 April to 14 May 2015

A stock solution with a nominal concentration of 100 mg ai/L was prepared prior to exposure initiation and solution renewal (day 7) by dissolving 0.3983 g of the test item completely in 3.8 L of test medium. Appropriate volumes of the stock solution were diluted to give the test concentration series. The control consisted of culture medium only.

3.5 L of the test solutions were transferred into 4 L glass flasks and inoculated with plants. Four replicate beakers for biological observations, each containing one pot with three plants, were established for each test concentration and six replicate beakers were established for the control, yielding 12 plants per test concentration and 18 plants for the control.

Plant health observations were performed at exposure initiation (day 0), on exposure day 7 and at exposure termination (day 14). Observations such as mortality, chlorosis, or necrosis were noted in the raw data, if present. After plants were harvested for biomass determination on day 14, visual observations of the roots were also made and any unusual findings were recorded.

At exposure termination (day 14), after biological observations, individual plants from each replicate were measured in length, then cut at the sediment surface. Each shoot was blotted dry, placed into a pre-weighed aluminium pan, and the pan was placed into a glass vessel covered with non-perforated plastic wrap until wet weight biomass was assessed later that same day. After wet weight biomass was assessed, the plants were dried in an oven at approximately 60 °C for a minimum of two days and individual shoot dry weights were determined using an analytical balance.

Instantaneous measurements of temperature, dissolved oxygen and pH in each test concentration and the control were recorded on exposure days 0, 7 and 14.

At exposure initiation (day 0), days 1, 3, 7 and 10 and exposure termination (day 14), an exposure solution sample was removed from each test concentration and the control for trinexapac-ethyl (parent), trinexapac (degradate) and CGA300405 (degradate) concentration determination. New solution samples, analyzed at days 0 and 7, were removed from the test and control solutions prior to division into the replicate test vessels. Aged solution samples, analysed at days 1, 3, 7, 10 and 14, were removed from the composited replicate solutions of each test concentration and control. At exposure initiation, day 7 and exposure termination, a sediment sample was removed from one of the additional replicates established for the 0.98, 3.1 and 10 mg/L treatment levels and the control. These samples were analyzed for trinexapac-ethyl (parent), trinexapac (degradate) and CGA300405 (degradate) concentration in pore water and sediment.

The test concentrations were verified by chemical analysis using liquid chromatography with tandem mass spectrometry detection (LC/MS/MS).

Results and Discussion

Measured concentrations of trinexapac-ethyl in newly prepared solutions (day 0 and day 7) and aged solutions (days 1, 3, 7, 10 and 14) maintained the expected concentration gradient. Exposure concentrations did decline over time, which was partially mitigated by solution renewal at day 7. At exposure initiation (day 0) and termination (day 14), concentrations ranged from 82 to 100% and 49 to 81% of nominal concentrations, respectively. The geometric mean measured concentrations ranged from 69 to 88% of nominal concentrations and defined the treatment levels tested as 0.025, 0.068, 0.26, 0.78, 2.6 and 8.8 mg/L of trinexapac-ethyl.

Measured concentrations of trinexapac increased over time in roughly the same proportion as the concomitant decrease in the parent (trinexapac-ethyl) concentration, which was partially mitigated by solution renewal at day 7. Measured concentrations of CGA300405 increased over time in the higher test concentrations, however, remained less than 1.00% of the parent concentrations. Trinexapac- concentrations at exposure initiation ranged from below the limit of quantitation (LOQ < 0.004 mg trinexapac-ethyl/L) to 0.079 mg trinexapac-ethyl/L (0.79%). Trinexapac concentrations at exposure termination ranged from 0.012 to 1.8 mg trinexapac-ethyl/L (18 to 40% respectively). CGA300405 concentrations were < LOQ at exposure initiation and ranged from < LOQ to 0.0083 mg trinexapac-ethyl/L (0.083%) at exposure termination. Since trinexapac and CGA300405 are degradates of trinexapac-ethyl and were not added to the test system, the presence of these degradates can be attributed solely to trinexapac-ethyl degradation during testing.

Measured pore water concentrations in the 0.98, 3.1 and 10 mg/L treatment levels were 0.0085, 0.13 and 0.040 mg/L, respectively, at exposure initiation, and were 0.029, 0.18 and 0.60 mg/L, respectively, at exposure termination.

Measured trinexapac pore water concentrations in the 0.98, 3.1 and 10 mg/L treatment levels were all < LOQ at exposure initiation, and were 0.16, 0.76 and 2.1 mg trinexapac-ethyl/L, respectively, at exposure termination. Measured CGA300405 pore water concentrations were < LOQ in all samples.

Measured sediment concentrations in the 0.98, 3.1 and 10 mg/L treatment levels were < LOQ (0.020 mg/kg), < LOQ and 0.048 mg/kg, respectively, at exposure initiation, and were 0.051, 0.21 and 0.57 mg/kg, respectively, at exposure termination.

Measured trinexapac sediment concentrations in the 0.98, 3.1 and 10 mg/L treatment levels were all < LOQ at exposure initiation, and were 0.073, 0.26 and 0.71 mg trinexapac-ethyl/kg, respectively, at exposure termination. Measured CGA300405 sediment concentrations were < LOQ in all samples at all test intervals.

The EC_{10}, EC_{20} and EC_{50} values were calculated, when possible, for 14-day total yield and average growth rate based on shoot length, shoot wet weight and shoot dry weight. EC values were calculated by linear interpolation of response (percent reduction of yield and growth rate compared to the control) versus the geometric mean measured concentration using the ICp method. For the No Observed Effect Concentration and Lowest Observed Effect Concentration, a Dunnett's Test was used to determine values significantly different to the control.

Mean growth rate based on shoot length is presented below along with growth and yield inhibition values:

Effect of trinexapac-ethyl on growth rate and yield of *Myriophyllum spicatum* for shoot length

Geometric mean measured concentration (mg/L)	Mean Final total shoot length	% Inhibition			
		Average specific growth rate		Yield (cm)	
		Mean (days-1)	Percent inhibition (%)	Mean (cm)	Percent inhibition (%)
Control	20.1	0.0945	-	14.7	-
0.025	16.5	0.0829	12	11.5	22
0.068	16.9	0.0846	10	11.8	20
0.26	14.8	0.0775	18	9.8*	33
0.78	11.1	0.0591*	37	6.3*	57
2.6	7.2	0.0226*	76	2.0*	87
8.8	5.9	0.0150*	84	1.1*	92

* Significantly reduced compared to the control, based on Dunnett's Multiple Comparison Test.

Mean wet weights are presented below along with the growth rate, yield and respective inhibition values:

Effect of trinexapac-ethyl on growth rate and yield (wet weight) of *Myriophyllum spicatum*

Geometric mean measured concentration (mg/L)	Shoot wet weight (g)	Shoot wet weight			
		Average specific growth rate		Yield (g)	
		Mean (days-1)	Percent inhibition (%)	Mean (g)	Percent inhibition (%)
Control	0.4767	0.1170	-	0.3848	-
0.025	0.3361	0.0902*	23	0.2442*	37
0.068	0.3157	0.0871*	26	0.2238*	42
0.26	0.2707	0.0767*	34	0.1787*	54
0.78	0.2671	0.0748*	36	0.1752*	54
2.6	0.1582	0.0365*	69	0.0663*	83
8.8	0.1190	0.0175*	85	0.0271*	93

* Significantly reduced compared to the control, based on Dunnett's Multiple Comparison Test.

Effect of trinexapac-ethyl on growth rate and yield (dry weight) of *Myriophyllum spicatum*

Geometric mean measured concentration (mg/L)	Shoot dry weight (g)	Shoot dry weight			
		Average specific growth rate		Yield (g)	
		Mean (days-1)	Percent inhibition (%)	Mean (g)	Percent inhibition (%)
Control	0.0331	0.0734	-	0.0213	-
0.025	0.0267	0.0569	22	0.0149	30
0.068	0.0244	0.0514*	30	0.0127*	41
0.26	0.0246	0.0520*	29	0.0128*	40
0.78	0.0280	0.0613#	17	0.0162#	24
2.6	0.0209	0.0389*	47	0.0091*	57
8.8	0.0214	0.0424	42	0.0096	55

* Significantly reduced compared to the control, based on Dunnett's Multiple Comparison Test.

Based on the effect observed at surrounding treatment levels (0.26 and 2.6 mg/L), this treatment level is considered a conservative NOEC.

Final results, EC10 EC20 EC50 NOEC LOEC values

Parameter	EC10	EC20	EC50	NOEC	LOEC
Yield (shoot length)	0.012 (95% c.i. 0.0042-0.19)	0.024 (95% c.i. 0.0084-0.36)	0.60 (95% c.i. 0.32-1.2)	0.068	0.26
Average growth (shoot length)	0.022 (95% c.i. 0.0076-0.49)	0.31 (95% c.i. n.d.-0.71)	1.2 (95% c.i. 0.63-1.8)	0.26	0.78
Yield (shoot wet weight)	0.0068 (95% c.i. 0.0038-0.039)	0.014 (95% c.i. 0.0075-0.057)	0.2 (95% c.i. n.d.-1.6)	<0.025	0.025
Average growth (shoot wet weight)	0.011 (95% c.i. 0.0053-0.055)	0.022 (95% c.i. 0.011-0.14)	1.4 (95% c.i. 0.64-2.4)	<0.025	0.025
Yield (shoot wet weight)	0.0083 (95% c.i. 0.0040-0.054)	0.017 (95% c.i. 0.0079-0.078)	1.9 (95% c.i. 0.58 - n.d.)	0.025	0.068
Average growth (shoot wet weight)	0.011 (95% c.i. 0.0057-0.059)	0.022 (95% c.i. 0.012-1.7)	>8.8 95% c.i. n.d.)	0.025	0.068

Conclusions

The analysis of the test solutions, pore water and sediment samples indicated that trinexapac-ethyl concentrations declined over time, which was partially mitigated by solution renewal at day 7. Additionally, these analyses indicated that as trinexapac-ethyl concentrations decreased, a corresponding increase occurred in concentrations of trinexapac and CGA300405. Since trinexapac and CGA300405 are degradates of trinexapac-ethyl and were not added to the test system, the presence of these degradates can be attributed solely to trinexapac-ethyl degradation during testing. The relative consistency of the results illustrate this degradation was a constant rate over the course of the testing and across test concentrations. Based on a comparison of 14-day EC_{50} values, yield for shoot wet weight produced the lowest EC_{50} value, 0.20 mg/L, and growth rate for shoot dry weight produced the highest EC_{50} value, > 8.8 mg/L.

<u>**Comments:**</u>

The validity criteria of OECD Guideline 239 were met:

- The growth multiple for shoot length in the control was 3.7; the growth multiple for shoot wet weight in the control was 5.2 (the mean total shoot length and mean shoot wet weight for the control must at least double during the exposure phase).

- The mean coefficient of variation for yield based on measurements of shoot wet weight in the control shoots must not exceed 35% between replicates. (Measured: 18%).
- The control plants were observed to be healthy throughout the exposure.

Consequently, the study is acceptable for use in risk assessment.

Endpoints were based on geometric mean measured concentrations of trinexapac-ethyl in the overlying water.

Measured sediment concentrations of both trinexapac-ethyl and trinexapac (CGA179500) were all at low levels at test termination and CGA300405 sediment concentrations were < LOQ at all sampling occasions.

Trinexapac-ethyl is not likely to persist in aquatic systems, including sediments. Its DT_{50} values have been calculated to be between 3.3 and 4.9 days for the water phase and its K_{FOC} (60 mL/g) indicates that the active substance will mostly be partitioned in the water column. This last point was validated by water sediment studies (Draft Assessment Report Volume 3, Annex B, B.8.6.3, February 2005), which showed that trinexapac-ethyl never reaches more than 6.0% AR in the sediment phase. In these laboratory water-sediment fate studies trinexapac-ethyl was shown to be rapidly degraded with the occurrence of the CGA179500 as the primary degradation product and eventually CO_2 as well as bound residues. Whilst the acidic component is slightly more persistent than the parent (DT_{50} in the total system 14 to 18 days), it has a similar K_{FOC} (140 mL/g) and a greater water solubility.

The lowest EC_{50} and NOEC results for algae or aquatic plants, the 14 day ErC_{50} of 1.2 mg a.s./L and NOEC of <0.025 mg a.s./L in *Myriophyllum spicatum* (Kirkwood, 2015), are carried forward for classification purposes.

2.9.2.3.4 Chronic toxicity to other aquatic organisms

No toxicity test with the sediment dwelling midge *Chironomus* spp. was deemed necessary for trinexapac-ethyl, trinexapac (CGA179500) or other metabolites, due to the short residence time of trinexapac-ethyl in the aquatic system and its moderate toxicity to *D.magna*. Also CGA179500 has low Kfoc (140 mL/g). The amount of the degradation product never reaches more than 6.9% AR in the sediment. Finally, the metabolites were shown to be of lower toxicity to aquatic organisms. No new data are provided. (For more information see volume 3-B.9 (AS)).

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

Table 74: Summary of information on acute aquatic toxicity relevant for classification

Method	Species	Test material	Results	Remarks	Reference
US EPA/FIFRA	Channel catfish (*Ictalurus punctatus*)	trinexapac-ethyl (purity	96 h-LC_{50} 35 mg a.s./L		Anonymous, (1991) CA8.2.1/04

Guideline 72-1		92.2%)	(mm)		CGA163935/0164
EPA Guideline No. 72-3	Bay shrimp (*Mysidopsis bahia*)	trinexapac-ethyl purity 92.2%)	96 h-LC_{50} 6.5 mg a.s./L (mm)		Sousa (1991) CGA163935_10634
OECD Guidelines 239 (2014)	***Myriophyllum spicatum***	**trinexapac-ethyl (95.4%)**	14 d shoot length **E_rC_{50} 1.2 mg a.s./L** Shoot wet wt E_rC_{50} 1.4 mg a.s./L shoot dry wt E_rC_{50} > 8.8 mg a.s./L (mm)	**(1.2 mg a.s./L**	**Kirkwood (2015) CGA163935_10672**
OECD 201 O.J. L383A, Part C.3: Algal inhibition test (1992) US EPA Guideline OPPTS 850.5400 Algal Toxicity, Tiers I and II, (1996)	Green alga *(Pseudokirchneriella subcapitata)*	trinexapac-ethyl (95.8%)	96 h E_rC_{50} 24.5 mg a.s./L (nom)		Cartee *et al.* (2009) CGA163935_10480

Toxicity tests were conducted for three trophic levels.

In aquatic toxicity studies the relevant (lowest) acute LC_{50} value for fish, EC_{50} value for aquatic invertebrates and ErC_{50} values for algae and aquatic macrophytes were all > 1mg/L. The lowest endpoint is 14 d shoot length ErC_{50} = 1.2 mg a.s./L for *Myriophyllum spicatum*.

The lowest relevant LC/EC_{50} value used in support of the active substance is the E_rC_{50} from testing with the aquatic plant *Myriophyllum spicatum*. The E_rC_{50} is 1.2 mg a.s./L. This is above the trigger for acute classification of 1.0 mg/L. Trinexapac-ethyl therefore is not classified as Aquatic Acute Cat.1

2.9.2.4.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Table 75: Summary of information on long-term aquatic toxicity relevant for classification

Method	Species	Test material	Results	Remarks	Reference
EPA guideline No. 72-4	Fathead minnow (*Pimephales promelas*)	trinexapac-ethyl (purity 92.2%)	35d-NOEC 0.41 mg a.s./L (mm)		Anonymous, (1991) CA8.2.2.1/01 CGA163935 /0189
US EPA/FIFRA Guideline 72-4	*Daphnia magna*	trinexapac-ethyl (93.8%)	21d-NOEC 2.4 mg a.s./L (mm)		**Putt (1994) CGA163935/0370**
OECD Guidelines 239 (2014)	***Myriophyllum spicatum***	**trinexapac-ethyl (95.4%)**	**NOEC <0.025 mg/L** (mm)	**<0.025 mg/L**	**Kirkwood (2015) CGA163935_10672**
OECD 201 O.J. L383A, Part C.3: Algal inhibition test (1992) US EPA Guideline OPPTS 850.5400 Algal Toxicity, Tiers I and II, (1996)	Green alga *(Pseudokirchneriella subcapitata)*	trinexapac-ethyl (95.8%)	96 h NOEC 8 mg a.s /L (nom)		Cartee *et al.* (2009) CGA163935_10480

In long-term toxicity studies NOEC values were > 0.1 mg/L for fish, aquatic invertebrates and algae. However, the chronic NOEC was < 0.1 mg L for aquatic macrophytes. The lowest NOEC is 14 d shoot dry wt. < 0.025 mg a.s./L for *Myriophyllum spicatum* (growth rate inhibition). According to the environmental fate data the active substance is classified as not readily biodegradable. As this lowest NOEC is less than 0.1 mg a.s./L and the substance is not readily biodegradable the classification Chronic category 1 (H410) 'very toxic to aquatic life with long lasting effects' is triggered. The related chronic M-factor is 1.

The Study (Baumann, W., 1993) was performed to determine the biodegradability of trinexapac-ethyl (purity 94.5%) in a carbon dioxide evolution test in activated sludge in accordance with the Guideline 92/69/EEC C.4-C, ready biodegradability carbon dioxide evolution test. Test was performed in duplicate with test media containing 26.9 and 27.9 mg test substance/L, equivalent to 16.6 and 17.2 mg theoretical organic carbon/L. Test was performed in 2 litre flasks which were connected to CO_2 traps. A reference substance of 15 mg DOC/L and a water control were included in the experiments. Measurements of the CO_2 content as inorganic carbon were performed with a carbon analyzer on the days 0, 3, 6, 8, 10, 15, 20, 24, 28 and 29.

Biodegradation of the test substance was 10% after 29 days and biodegradation of the reference was 87% after 29 days.

The results of the test on biodegradation of trinexapac-ethyl show that trinexaoac-ethyl is considered not rapidly degradable (a degradation > 70% within 28 days) for purpose of classification and labelling

Trinexapac-ethyl does not have potential to bioaccumulate, The octanol - water partition coefficient of trinexapac-ethyl is pH-dependent and at environmentally relevant pH-values of approximately 7, trinexapac-ethyl has a log P_{ow} below 3 (pH 6.9 log P_{ow} = -0.29). The experimentally derived steady state BCF of 6 L/kg ww for trinexapac-ethyl related to total radioactivity whole fish is lower than the trigger of 500 (criterion for bioaccumulation potential conform Regulation EC 1272/2008)

2.9.2.5 Conclusion on classification and labelling for environmental hazards

The acute LC_{50} and EC_{50} values for aquatic organisms are above 1 mg a.s./L. therefore, Trinexapac ethyl is not classified as Aguatic Acute cat.1.

The chronic NOEC values for aquatic organisms are below 0.1 mg a.s./L. Trinexapac-ethyl is not considered to rapidly degrade and does not meet the criteria for a potential to bioaccumulate. Therefore, the CLP classification for chronic aquatic hazard is Category Chronic 1.

Based on ther lowest endpoint, the NOEC is 0.025 mg a.s./L, derived from the Myriophyllum spicatum study. The chronic NOEC value of 0.025 mg a.s./L is between 0.01 and 0.1 mg/L, therefore a M-factor of 1 is applied, based on non-rapidly degradable components.

In conclusion:

Acute aquatic hazard: Not classified

Long term aquatic hazard: Aquatic Chronic category 1, M-factor; 1

2.9.3 Summary of effects on arthropods

The toxicity of trinexapac-ethyl and A8587F to honey-bees has been investigated by carrying out acute adult, chronic adult and larval development laboratory toxicity studies.

Data on the acute oral and contact toxicity of the active substance tinexapac –ethyl to bees were previously submitted and evaluated in the context of the original EU review of this active substance. Data were considered acceptable and no further studies were considered necessary in relation to the first approval of trinexapac-ethyl.

However, for purposes of completeness new data on acute and oral toxicity for active substance and for representative formulation were submitted and were used in the risk assessment as the lowest available endpoints.

In support of the AIR application new data on chronic effects of representative formulation *in lieu* of the technical active substance were generated in view of new data requirements se in in the Annex to Commission Regulation 283/2013.

Acute oral and contact LD_{50} values for adult acute exposure were >83 μg a.s./bee and >100 μg a.s./bee. The larval NOED was found to be 83.4 µg a.s./larva and the chronic adult NOED was 26.9 µg a.s./bee/day.

The toxicity of A8587F to non-target arthropods has been investigated by carrying out both Tier I (glass plate) (the resultant LR_{50} values were >60 <80 ml/ha) and Tier II (extended laboratory) tests on the sensitive indicator species *Aphidius rhopalosiphi* and *Typhlodromus pyri* with A8587B (the A8587F equivalent formulation). The resultant LR_{50} values were LR_{50} >3000 ml/ha. To further support the risk assessment, additional Tier II (extended laboratory) tests with formulation A8587F have also been carried out with *Orius insidiosus* and *Chrysoperla carnea*. The resultant LR_{50} values were LR_{50} >3000 ml/ha. These four species are tested, in accordance with ESCORT 2, as representative non-target arthropods since they have been found to be particularly sensitive species, and therefore can be considered as indicators of potential effects to the most sensitive non-target arthropods in the field.

2.9.4 Summary of effects on non-target soil meso- and macrofauna

Data on acute toxicity of the active substance trinexapac-ethyl and its metabolite trinexapac were previously submitted and evaluated in the context of the original EU review. The results from these studies demonstrate that trinexapac-ethyl ant its metabolite is of low acute toxicity to earthworms.

Acute earthworm studies are no longer a data requirement and are not incorporated into the soil organism risk assessment. . Since submission new studies with the metabolites trinexapac (CGA179500) and CGA300405 has been completed, these new studies are made available for consideration. The earthworms NOEC was found to be 24.3 mg CGA179500/kg dry soil and 1000 mg CGA300405/kg dry soil.

A new study has been carried out for trinexapac-ethyl on *Eisenia fetida* to fulfil current data requirements in Commission Regulation (EU) No 283/2013. The study has been carried out with the representative formulation, A8587F, *in lieu* of the technical active substance and has been submitted addressing the risk to soil organisms from exposure to the formulated active substance. The long-term toxicity of trinexapac-ethyl to earthworms (NOEC 309 mg formulation/kg (81.9 mg a.s./kg)). Also studies were provided demonstrating toxicity to soil macro-organisms *Hypoaspis aculeifer* and *Folsomia candida* from the representative formulation and metabolite CGA300405. *F.candida* 28-day NOEC = 95 mg form/kg dry soil, *H.aculeifer* 14-day NOEC = 95 mg form/kg dry soil and NOEC = 1000 mg CGA300405/kg dry soil.

2.9.5 Summary of effects on soil nitrogen transformation

In the original EU review of trinexapac-ethyl study on effects of technical material on soil microorganisms was submitted. Effects on nitrogen transformation and carbon mineralisation of trinexapac-ethyl applied to soil were evaluated and accepted.

The toxicity of A8587F with the equivalent formulation, A8587B + 0.1% Extravon (A4218A), and trinexapac-ethyl to soil micro-organisms was provided. No separate test has been performed with the major soil metabolite trinexapac (CGA179500) since its possible effects are considered to be covered by the test with the parent compound due to the rapid conversion of trinexapac-ethyl into trinexapac in viable soils. Since submission a new study with the metabolite CGA300405 has been completed. After 28-days no effect >25% on nitrification and respiration were seen at 2.6 mg a.s./kg dry soil and no effect >25% on nitrification and respiration were seen at

200 mg CGA300405/kg dry soil.

2.9.6 Summary of effects on terrestrial non-target higher plants

In the original EU review of trinexapac-ethyl study on effects of technical material on seedling emergence and vegetative vigour was submitted. Effects on pre-and post-emergence non-target higher plants were evaluated and accepted. The lowest endpoints were seedling emergence ER_{50} > 0.84 (kg a.s./ha) and vegetative vigour ER_{50} > 0.76 (kg a.s./ha).

Tier I non-GLP studies on pre- and post-emergence non-target higher plants conducted on A8587F, and the equivalent formulation A8587B were provided. The lowest endpoints were seedling emergence ER_{50} > 0.38 (kg a.s./ha) and vegetative vigour ER_{50} > 0.38 (kg a.s./ha).

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

An acute study on the frog (*Xenopus laevis*) has been conducted with the technical active substance (Ding, Q., 2008, Syngenta File No. CGA163935_10559), to fulfil data requirements in China. The 48 hour LC_{50} was >106 mg /L which is greater than the existing aquatic acute vertebrate data with fish.

2.9.8 Summary of effects on biological methods for sewage treatment

In the study presented for the first annex I inclusion the respiration rate (oxygen consumption) of an aerobic activated sludge fed with a standard amount of synthetic sewage was measured in the presence of 100 mg a.s./L after an incubation period of 3 hours. Under the conditions of this study, trinexapac-ethyl had no toxic effect on activated sludge up to at least the limit test concentration of 100 mg a.s./L.

Two additional studies have been carried out for trinexapac-ethyl on activated sludge respiration. Based on the newly submitted studies a 3 h EC_{50} of 100 and 1000 mg a.s./L were determined respectively.

2.9.9 Summary of product exposure and risk assessment

Birds

Risk assessment for birds from the critical uses proposed for A8587F has been carried out according to the latest draft of the ‘EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).

Table 2.9.9-1: Screening step - Acute risk (TER_A) to birds from trinexapac-ethyl

Test item	Crop group	Indicator species	LD_{50} (mg a.s./kg bw)	DDD (mg a.s./kg bw/day)	TER_A	Trigger value
Trinexapac-ethyl	cereal	Small omnivorous bird	>2000	31.8	>63	10

The TER_A value for trinexapac-ethyl for the indicator species is greater than the trigger of 10, indicating that the acute risk to birds is acceptable following use of A8587F according to the proposed use pattern is acceptable.

Table 2.9.9-2: Screening step – long-term (TER_{LT}) to birds from trinexapac-ethyl

Test item	Crop group	Indicator species	NOEL (mg a.s./kg bw/day)	DDD (mg a.s./kg bw/day)	TER_{LT}	Trigger value
Trinexapac-ethyl	cereal	Small omnivorous bird	17.6	6.87	2.6	5

The TER_{LT} value for trinexapac-ethyl for the indicator species is lower than the trigger of 5, indicating that the long-term risk to birds following use of A8587F according to the proposed use pattern is unacceptable. Further refinement is thus needed for this use.

Reproductive risk assessment for birds-Tier 1 risk assessment

Table 2.9.9.1-3: Tier 1 – estimates of long-term exposure and risk to trinexapac-ethyl following application of Trinexapac-ethyl 250 g/L ME in cereals

Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg a.s./ha)	MAF	f_{TWA}	DDD (mg a.s./kg bw/ day)	NOEL (mg a.s./kg bw/day)	TER_{LT}	Trigger value
Cereals Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	16.2	0.2	1	0.53	1.72	17.6	10	5
Cereals BBCH 10-29	Small omnivorous bird "lark	10.9				1.16		15	
Cereals BBCH 30-39	Small omnivorous bird "lark	5.4				0.6		29	
Cereals BBCH ≥ 40	Small omnivorous bird "lark	3.3				0.35		50	

The TER values calculated in the above first tier reproductive risk assessment for birds are in excess of the Annex VI trigger value of 5. Thus, the reproductive risk to birds can be concluded a low for the representative uses on winter and spring cereals.

Mammals

Risk assessment for mammals from the critical uses proposed for A8587F has been carried out according to the latest draft of the 'EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).

Table 2.9.9-4: Screening step - Acute risk (TER_A) to mammals from trinexapac-ethyl

Compound	Crop group	Indicator species	LD_{50} (mg a.s./kg bw)	DDD (mg a.s./kg bw/day)	TER_A	Trigger

Compound	Crop group	Indicator species	LD_{50} (mg a.s./kg bw)	DDD (mg a.s./kg bw/day)	TER_A	Trigger
Trinexapac-ethyl	cereal	Small herbivorous mammal	4210	23.7	178	10
A8587F	cereal	Small herbivorous mammal	>750	23.7	>32	10

The TER_A values for trinexapac-ethyl and A8587F for the indicator species are greater than the trigger of 10, indicating that the acute risk to mammals following use of A8587F according to the proposed use pattern is acceptable.

Table 2.9.9-5: Screening step - long-term risk (TER_{LT}) to mammals

Compound	Crop group	Indicator species	NOEL (mg a.s./kg bw/day)	DDD (mg/a.s./kg bw/day)	TER_{LT}	Trigger
Trinexapac-ethyl	cereals	Small herbivorous mammal	60	5.12	12	5

The TER_{LT} value for trinexapac-ethyl is higher than the trigger value of 5, indicating that the long-term risk to mammals following use of A8587F according to the proposed use pattern is acceptable.

Aquatic organisms

The risk assessment for effects on aquatic organisms has been conducted according to the EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013). For the exposure and risk assessment, the single application (for winter cereals) at a rate of 200g a.s./ha was considered for the representative uses of the formulation Trinexapac-ethyl 250g/L ME in cereals.

Formulated product A8587F

The results from the toxicity tests using the A8587F formulation indicate that the toxicity of the formulation reflects the toxicity of the constituents and hence the risk assessments are conducted with the active substance toxicity endpoints, as shown in the tables below.

Table 2.9.9-6: Trinexapac-ethyl RAC values

Organism group	Test organism	Endpoint		AF	Tier 1-RAC
		(type)	(µg/L)		(µg/L)
Acute effects					
Fish (Pisces Ictaluridae)	Channel Catfish *Ictalurus punctatus*	96 hr LC_{50}	35 000	100	350
Aquatic invertebrates (Mysidae)	Bay shrimp (*Mysidopsis bahia*)	96 hr LC_{50}	6 500		65
Chronic effects					
Fish (Pisces, Cyprinidae)	*Pimephales promelas*	Early life stage 35 d NOEC	410	10	41
Aquatic invertebrates (Crustacea, Daphniidae)	*Daphnia magna*	21 d NOEC	2400		240
Green algae	*Pseudokirchneriella*	96 h E_rC_{50}	24 500		2 450

Organism group	Test organism	Endpoint		AF	Tier 1-RAC
		(type)	(µg/L)		(µg/L)
	subcapitata				
Aquatic macrophyte	*Lemna gibba*	7 d E_bC_{50}	8 800		880
Aquatic macrophyte	Eurasian watermilfoil *Myriophyllum spicatum*	14 d E_yC_{50}	200		20

Value in **bold** is considered as Tier 1-RAC

Table 2.9.9-7: Trinexapac (CGA179500) RAC values

Organism group	Test organism	Endpoint		AF	Tier 1-RAC
		(type)	(µg/L)		(µg/L)
Acute effects					
Fish (Pisces salmonidae)	Rainbow trout (*Oncorhynchus mykiss*)	96 hr LC_{50}	>100 000	100	>1 000
Fish (Pisces, Cyprinidae)	Common carp *Cyprinus carpio*	96 hr LC_{50}	>100 000		>1 000
Aquatic invertebrates (Crustacea, Daphniidae)	*Water flea (Daphnia magna)*	48 hr EC_{50}	>111 000		>1 110
Chronic effects					
Blue green alga	*Anabaena flos-aquae*	72 h E_rC_{50}	20 100	10	2010
Aquatic macrophyte	*Lemna gibba*	7 d E_bC_{50}	1 500		150

Value in **bold** is considered as Tier 1-RAC

Table 2.9.9-8: CGA300405 RAC values

Organism group	Test organism	Endpoint		AF	Tier 1-RAC
		(type)	(µg/L)		(µg/L)
Acute effects					
Aquatic invertebrates (Crustacea, Daphniidae)	*Water flea (Daphnia magna)*	48 hr EC_{50}	>100 000	100	>1 000
Chronic effects					
Green algae	*Pseudokirchneriella subcapitata*	96 h E_rC_{50}	>100 000	10	>10 000
Aquatic macrophyte	*Lemna gibba*	7 d E_rC_{50}	>100 000		>10 000

Value in **bold** is considered as Tier 1-RAC

Risk assessment A8587F

Table 2.9.9-9: A8587F – Comparison of Tier1-RAC and PEC_{SW} values

Parameter	Organism group	Endpoint (µg/L)	Tier 1-RAC (µg/L)	Initial PEC_{SW} (µg/L)
Acute exposure	Fish	94 000	940	6.99

The relevant organism is *Oncorhynchus mykiss* with a Tier 1-RAC_{ac} of 940 µg/L. The maximum initial PEC value in spring cereals and winter cereals is 6.99 µg A8587F/L which is below the acute RAC value for fish, indicating acceptable risk.

Risk assessment trinexapac-ethyl

Table 2.9.9-10: Trinexapac-ethyl – Comparison of Tier1-RAC and PEC_{SW} values

Parameter	Organism group	Tier 1-RAC	Max PEC_{SW} (Focus Step 2)
		(µg/L)	(µg/L)
Acute exposure	Aquatic invertebrates (Mysidae)	65	1.84
Chronic exposure	Aquatic macrophyte	20	1.84

The most sensitive organisms are mysid shrimp and *Myriophyllum* with an acute RAC_{ac} of 65 µg/L and chronic RAC of 20 µg/L respectively. The maximum FOCUS step 2 value in spring cereals and winter cereals is 1.84 µg/L which is below the acute and chronic RAC value for fish, aquatic invertebrates, alga and macrophytes indicating acceptable risk.

Risk assessment trinexapac (CGA179500)

Table 2.9.9-11: Trinexapac (CGA179500) – Comparison of Tier1-RAC and PEC_{SW} values

Parameter	Organism group	Tier 1-RAC	Max PEC_{SW} (Focus Step 2)
		(µg/L)	(µg/L)
Acute exposure	Fish (Cyprinidae & Salmonidae)	>1000	12.1
Chronic exposure	Aquatic macrophyte	150	12.1

The most sensitive organisms are fish, aquatic invertebrates and then *Lemna*, with an acute RAC of >1000 µg/L and chronic RAC of 150 µg/L. The maximum FOCUS step 2 value in spring cereals and winter cereals is 12.1µg/L for Northern Europe and 9.86µg/L for Southern Europe; both these values are below the acute and chronic RAC value for fish, aquatic invertebrates, alga and macrophytes indicating acceptable risk.

Risk assessment CGA300405

Table 2.9.9-12: CGA300405 – Comparison of Tier1-RAC and PEC_{SW} values

Parameter	Organism group	Tier 1-RAC	Max PEC_{SW} (Focus Step 2)
		(µg/L)	(µg/L)
Acute exposure	Aquatic invertebrates (Daphniidae)	>1000	0.61
Chronic exposure	Green algae	3 300	0.61

The most sensitive organisms are aquatic invertebrates and then algae (green algae), with an acute RAC of >1000 µg/L and chronic RAC of 3300 µg/L. The maximum FOCUS step 2 values in spring cereals and winter cereals is 0.61µg/L for Northern Europe and 1.05 µg/L for Southern Europe; both these values are below the acute and chronic RAC value for aquatic invertebrates, alga and macrophytes indicating acceptable risk.

Risk Assessment M2

Table 2.9.9-13: M2 – Comparison of Tier1-RAC and PEC_{SW} values

Parameter	Organism group	Tier 1-RAC[a]	Max PEC_{SW} (Focus Step 2)
		(µg/L)	(µg/L)
Acute exposure	Aquatic invertebrates (Mysidae)	6.5	0.38
Chronic exposure	Aquatic macrophyte	2.0	0.38

[a] worst-case Tier 1 RAC are estimated based on toxicity of parental compound trinexapac-ethyl under the assumption that metabolites are up to 10 times more toxic than parental compound

The worst-case Tier 1 RAC for M2 is estimated based on toxicity of parental compound trinexapac-ethyl under the assumption that metabolites are up to 10 times more toxic than parental compound. The most sensitive organisms are mysid shrimp and *Myriophyllum* with an acute RAC_{ac} of 6.5 µg/L and chronic RAC of 2.0 µg/L respectively. The maximum PECsw values derived from parent PECsw in spring cereals and winter cereals is 0.38 µg/L which is below the acute and chronic RAC value for fish, aquatic invertebrates, alga and macrophytes indicating acceptable risk.

Risk assessment WaterM3Photolysis

Table 2.9.9-14: WaterM3Photolysis – Comparison of Tier1-RAC and PEC_{SW} values

Parameter	Organism group	Tier 1-RAC[a]	Max PEC_{SW} (Focus Step 2)
		(µg/L)	(µg/L)
Acute exposure	Aquatic invertebrates (Mysidae)	6.5	0.31
Chronic exposure	Aquatic macrophyte	2.0	0.31

[a] worst-case Tier 1 RAC are estimated based on toxicity of parental compound trinexapac-ethyl under the assumption that metabolites are up to 10 times more toxic than parental compound

The worst-case Tier 1 RAC for WaterM3Photolysis is estimated based on toxicity of parental compound trinexapac-ethyl under the assumption that metabolites are up to 10 times more toxic than parental compound. The most sensitive organisms are mysid shrimp and *Myriophyllum* with an acute RAC_{ac} of 6.5 µg/L and chronic RAC of 2.0 µg/L respectively. The maximum PECsw values derived from parent PECsw in spring cereals and winter cereals is 0.31 µg/L which is below the acute and chronic RAC value for fish, aquatic invertebrates, alga and macrophytes indicating acceptable risk.

Risk assessment CGA275537

Table 2.9.9-15: CGA275537 – Comparison of Tier1-RAC and PEC_{SW} values

Parameter	Organism group	Tier 1-RAC[a]	Max PEC_{SW} (Focus Step 2)
		(µg/L)	(µg/L)
Acute exposure	Aquatic invertebrates (Mysidae)	6.5	<0.001
Chronic exposure	Aquatic macrophyte	2.0	<0.001

[a] worst-case Tier 1 RAC are estimated based on toxicity of parental compound trinexapac-ethyl under the assumption that metabolites are up to 10 times more toxic than parental compound

The worst-case Tier 1 RAC for CGA275537 is estimated based on toxicity of parental compound trinexapac-ethyl under the assumption that metabolites are up to 10 times more toxic than parental compound. The most sensitive organisms are mysid shrimp and *Myriophyllum* with an acute RAC_{ac} of 6.5 µg/L and chronic RAC of 2.0 µg/L respectively. The maximum PECsw values derived from parent PECsw in spring cereals and winter cereals is <0.001 µg/L which is below the acute and chronic RAC value for fish, aquatic invertebrates, alga and macrophytes indicating acceptable risk.

Conclusion: **the risk of trinexapac-ethyl and its metabolites to aquatic organisms from the intended use of the formulation Trinexapac-ethyl 250 g/L ME in cereals is acceptable without risk mitigation measures.**

Bees

Acute risk assessment

The potential acute and chronic risk from use of A8587F was assessed in accordance with the current Terrestrial Guidance Document, EPPO 2010 scheme and also according to EFSA document on bees (2013).

Oral exposure Q_{HO}

Table 2.9.9-16: Risk to bees from oral exposure to A8587F

Test item	**Application rate (g a.s./ha)**	**Oral LD_{50} (µg/bee)**	**Hazard quotient**	**Trigger**
Trinexapac-ethyl (as formulation A8587F)	200	>104 µg a.s./bee	<1.92	50
Trinexapac-ethyl	200	>83 µg a.s./bee	<2.41	

The hazard quotients for trinexapac-ethyl formulated as A8587F are less than 50, indicating that the risk to bees following use of A8587F according to the proposed use pattern is acceptable.

Contact exposure Q_{HC}

Table 2.9.9-17: Risk to bees from contact exposure to A8587F

Test item	**Application rate (g a.s./ha)**	**Contact LD_{50} (µg/bee)**	**Hazard quotient**	**Trigger**
Trinexapac-ethyl (as formulation A8587F)	200	168 µg a.s./bee	1.19	50
Trinexapac-ethyl	200	>100 µg a.s./bee	<0.50	

The hazard quotients for trinexapac-ethyl formulated as A8587F are less than 50, indicating that the risk to bees following use of A8587F according to the proposed use pattern is acceptable.

Plant metabolites - To assess the risk to bees from metabolites we can conservatively assume that the metabolites are 10 times more toxic that the parent. If this were the case and the exposure conservatively assumed to be 200 g/ha then the hazard quotients would be 10 times those shown in tables 2.9.9.3.1-2 and 4. That would give values of <19.2, <24.1, 11.9 and <5.0. In all cases these hazard quotients are less than 50, indicating that the risk to bees is acceptable.

Chronic Risk Assessment

Chronic adult and larval bee studies have been conducted according to the data requirements under 1107/2009. The endpoints from these studies have been assessed by adapting the EPPO 2010 scheme. The risk assessment indiceted an acceptable risk to to bee larval development and an acceptable chronic risk to adult bees

The risk assessment to honeybees also has been performed (first tier) according to EFSA document on bees (2013).

Table 2.9.9-18: Screening step – Risk assessment of chronic oral exposure to trinexapac-ethyl

Test substance	Application Category	Crop Group	Species	App. rate (kg a.s./ha)	Shortcut Value (downward spray)	LDD_{50} oral (µg a.s./bee/day)	$ETR_{chronic\ adult\ oral}$	Trigger
Trinexapac-ethyl (as formulation A8587F)	Downward Spray	Cereals	Honeybee	0.200	7.55	46.6	**0.032**	0.030

HQ/ETRs in **bold** are above the relevant trigger and require further refinement

The sceening step **ETR$_{\textbf{chronic adult oral}}$** value of 0.32 for trinexapac-ethyl in cereals is slightly greater than the trigger of 0.03 for downward sprays, according to EFSA 2013, indicating a need for further refinement. This is given in the table below.

Table 2.9.9-19: First tier risk assessment for chronic exposure (Cereals BBCH 25-49)

Scenario	App. rate (kg a.s./ha)	Ef	SV	TWA	LDD_{50} oral (µg a.s./bee/day)	$ETR_{chronic\ adult\ oral}$	Trigger
Crop	0.200	1	0.92	0.72	46.6	0.0028	0.0300
Weeds		1	2.9	0.72		0.0090	
Field margin		0.092	2.9	0.72		0.0008	
Adjacent crop		0.003	5.8	0.72		0.00005	
Next crop		1	0.54	0.72		0.0017	

HQ/ETRs in **bold** are above the relevant trigger and require further refinement

The tier 1 **ETR$_{\textbf{chronic adult oral}}$** values for trinexapac-ethyl are all less than the trigger of 0.03 for downward sprays, according to EFSA 2013, indicating that the risk to honeybee larvae is acceptable following use of A8587F according to the proposed use pattern.

Plant metabolites - To assess the risk to bees from metabolites we can conservatively assume that the metabolites are 10 times more toxic that the parent. If this were the case and the exposure conservatively assumed to be 200 g/ha then the ETR values would be 10 times those shown in Table 2.9.9.3.1-4, this still gives values that are well below the trigger of 0.03.

EFSA Larval assessment

Table 2.9.9-20: Screening step – Risk assessment of larval exposure to trinexapac-ethyl

Test substance	Application Category	Crop Group	Species	App. rate (kg a.s./ha)	Shortcut Value (downward spray)	NOED oral (µg a.s./larva/ development period)	ETR_{larvae}	Trigger
Trinexapac-ethyl	Downward Spray	Cereals	Honeybee	0.200	4.4	12.6	0.070	0.200

HQ/ETRs in **bold** are above the relevant trigger and require further refinement

The **ETR$_{\textbf{larvae}}$** value for trinexapac-ethyl is less than the trigger of 0.2 for downward sprays, according to EFSA 2013, indicating that the risk to honeybee larvae is acceptable following use of A8587F according to the proposed use pattern.

Plant metabolites - To assess the risk to bees from metabolites we can conservatively assume that the metabolites are 10 times more toxic that the parent. If this were the case and the exposure conservatively assumed to be 200 g/ha then the ETR value is 0.7 and requires tier 1 refinement, this is given below.

Table 2.9.9-21: First tier risk assessment plant metabolites

Scenario	App. rate (kg a.s./ha)	Ef	SV	TWA	NOED	ETR	Trigger
Crop	0.200	1	0.15	0.85	1.26	0.02	0.20
Weeds		0.5	2.2	0.85		0.15	
Field margin		0.092	2.2	0.85		0.03	

HQ/ETRs in **bold** are above the relevant trigger and require further refinement

These ETR values are all less than the trigger of 0.2, indicating that the risk to honeybee larvae is acceptable.

EFSA Contaminated water risk assessment

The ETR values for contaminated water are calculated as follows:

Acute

ETRacute = W * PEC/LD50

where W = 11.4 µL/bee per day and is the uptake of adult bees. Where the PEC is the concentration in the guttation water in µg/µL and is assumed to be 100% of the water solubility for the acute risk assessment in the first tier (see Appendix T). The LD50 is the oral LD50 in µg per adult bee.

ETRacute = (11.4 x 0.0011)/83 = 0.00015

The subsequent ETR is considered to demonstrate acceptable risk where it is less than the applicable trigger value of 0.2.

Chronic

ETRchronic = W * PEC/LD50

where W = 11.4 µL/bee per day and is the uptake of adult bees. Where the PEC is the concentration in the guttation water in µg/µL and is assumed to be 100% of the water solubility and the LD50 is the 10 day LD50 in µg per adult bee.

ETRchronic = (11.4 x 0.0011)/46.6 = 0.00027

The subsequent ETR is considered to demonstrate acceptable risk where it is less than the applicable trigger value of 0.03

Larval

ETRchronic = W * PEC/NOEC

where W = 111 µL/bee per day and is the uptake of adult bees. Where the PEC is the concentration in the guttation water in µg/µL and is assumed to be 100% of the water solubility and the NOEC.

ETRchronic = (111 x 0.0011)/12.6 = 0.0097

The subsequent ETR is considered to demonstrate acceptable risk where it is less than the applicable trigger value of 0.2

All of these ETR values for contaminated water are less than the trigger values for downward sprays, indicating that the risk to honeybee larvae is acceptable following use of A8587F according to the proposed use pattern.

The screening $ETR_{chronic\ adult\ oral}$ is below the relevant trigger, indicating a need for further refinement. In the refinement -1[nd] tier, all the ETRchronic adult oral are below the relevant trigger, indicating an acceptable chronic risk to adult bees.

Furthermore, it should be noted that chronic effects on bees and larvae are unlikely since exposure to residues of trinexapac-ethyl from intended use of the formulation trinexapac-ethyl 250 g/L ME in cereals is limited. Cereals are not considered attractive for bees as a source of food (pollen and nectar). Weeds present in cereals fields might be attractive to bees, however usually it is not expected high presence of flowering weeds in cereals fields. Therefore taking into account all available information it can be considered that exposure to bees is unlikely and that the acute and the chronic risk is considered acceptable.

Arthropods other than bees

The risk to non-target arthropods is assessed using the approach recommended in the published ESCORT 2 document (**Candolfi et al. 2001**) and the **EC Guidance Document on Terrestrial Ecotoxicology**.
The exposure of non-target arthropods to the formulation A8587F, expressed as Predicted Environment Rate (PER) was assessed separately for the in-field and off-field area.

In-field

Table 2.9.9-22: In-field Tier 1 HQs for non-target arthropods

Species	**LR_{50} (mL/ha)**	**In-field foliar**		**In-field soil**		**Trigger value**
		PER (mL/ha)	HQ	PER (mL/ha)	HQ	
Aphidius rhopalosiphi Tier I, 2D exposure scenario (limit test)	>610[a]	800	<1.3	800	<1.3	2
Aphidius rhopalosiphi Tier I, 2D exposure scenario (3 test rates)	>60[a]	800	<13	800	<13	2
Typhlodromus pyri Tier I, 2D exposure scenario (3 test rates)	>60[a]	800	<13	800	<13	2

[a] Due to the limited number of rates tested, conservative values have been used which are considered to underestimate the LR_{50}.

The in-field HQ values for both *A. rhopalosiphi* and *Typhlodromus pyri* are above the trigger value of 2, indicating the need for further evaluation of the potential risk to in-field non-target arthropods. In order to address this potential risk, additional assessments based on extended laboratory data are presented below.
For higher tier studies, a trigger value of 50% effect on lethal or sublethal endpoints is employed. If the LR_{50}, or sublethal 50% effect value is greater than or equal to the PER value then no unacceptable effects would be predicted in-field following the use of A8587F in accordance with the uses supported in this submission.
The in-field assessment is presented in the table below.

Table 2.9.9-23: In-field risk assessment for non-target arthropods

Test species	Endpoints (mL A8587F/ha)		Soil		Foliage	
			PER (mL/ha)	Acceptable risk	PER (mL/ha)	Acceptable risk
T. pyri	LR_{50}	>3000	800	Yes	800	Yes
	NOER (reproduction)	3000				
A. rhopalosiphi	LR_{50}	>3000	800	Yes	800	Yes
	NOER (reproduction)	3000				
O. insidiosus	LR_{50}	>3000	800	Yes	800	Yes
	NOER (reproduction)	3000				
C. carnea	LR_{50}	>3000	800	Yes	800	Yes
	NOER (reproduction)	3000				

The LR_{50} and NOER endpoints for all species tested in the extended laboratory studies are greater than the PER values indicating an acceptable risk to non-target arthropods. Furthermore, no effects on fecundity greater than 50% were observed for any of the species at rates up to 3000 mL A8587F/ha confirming that A8587F poses an acceptable in-field risk to non-target arthropods.

<u>Off-field</u>

The off-field assessment, calculated according to ESCORT 2, is presented in the table below.

Table 2.9.9-24: Off-field risk assessment for non-target arthropods

Test species	Endpoints (mL A8587F/ha)		PER[a] (mL/ha)	Acceptable risk
T. pyri	LR_{50}	>3000	11.1	Yes
	NOER (reproduction)	3000	11.1	Yes
A. rhopalosiphi	LR_{50}	>3000	111	Yes
	NOER (reproduction)	3000	111	Yes
Orius insidiosus	LR_{50}	>3000	11.1	Yes
	NOER (reproduction)	3000	11.1	Yes
Chrysoperla carnea	LR_{50}	>3000	11.1	Yes
	NOER (reproduction)	3000	11.1	Yes

[a] This represents the off-field PER given in Table 10.3.2-4 multiplied by the correction factor of 5, as recommended by ESCORT2 guidance.

The LR_{50} and NOER endpoints for all species tested are greater than the PER values, confirming an acceptable risk to non-target arthropods following the use of A8587F according to the proposed GAP; no further evaluation is considered necessary.

Conclusion: the risk to non-target terrestrial arthropods is considered acceptable for the intended use of the formulation Trinexapac-ethyl 250 g/L ME in cereals. Taking into account higher tier data an acceptable risk at the maximum intended application rate is demonstrated.
Furthermore, since the HQ values at 1 m off-crop distance show an acceptable risk, the potential for recovery of the in-crop population by immigration and recolonisation can be expected if the in-crop population would be affected. No risk mitigation measures are therefore required.

Earthworms

The risk assessment for effects on non-target soil meso-and macrofauna has been conducted according to the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10239/2002) The potential long-term risk of trinexapac-ethyl was assessed by calculating long-term TER (TER_{LT}) values by comparing the NOEC values and the PEC_S.

Table 2.9.9-25: Long-term TER values for earthworms

Test substance	NOEC (mg/kg soil)	PEC_S (mg/kg soil)	TER_{LT}	Trigger value
A8587F	309 mg formulation/kg	0.807	383	5
Trinexapac-ethyl	81.9 mg a.s./kg	0.213	385	
Trinexapac (CGA179500)	NOEC: 24.3 mg CGA179500/kg soil	0.179	136	
CGA300405	NOEC: 1000 mg CGA300405/kg soil	0.022	45 454	
CGA275537[c]	8.19 mg a.s./kg	0.016	512	

[c] It is asummed that metasbolites are up to 10 times more toxic than parental compound trinexapac-ethyl.

The long-term TER values exceed the Commission Regulation (EU) No. 546/2011 long-term trigger value of 5, indicating that the long-term risk to earthworms is acceptable following use of A8587F according to the proposed use pattern.

Non-target soil meso- and macrofauna (other than earthworms)

The potential long-term risk of trinexapac-ethyl to other non-target soil meso- and macro-fauna was assessed by calculating long-term TER (TER_{LT}) values by comparing the NOEC values and the maximum instantaneous PEC_S.

Table 2.9.9-26: Long-term TER values for other soil meso- and macro-fauna

Organism	Test substance	NOEC (mg/kg soil)	PEC_S (mg/kg soil)	TER_{LT}	Trigger value
Folsomia candida	A8587F	95 mg/kg	0.807	118	5
	Trinexapac-ethyl	25.2 mg a.s./kg dw soil	0.213	118	
Hypoaspis aculeifer	A8587F	95 mg/kg	0.807	118	
	Trinexapac-ethyl	25.2 mg a.s./kg dw soil	0.213	118	
Folsomia candida *Hypoaspis aculeifer*	Trinexapac (CGA179500) [a]	Alternatively an estimated NOEC for 2.52 mg/kg soil	0.179	14	
	CGA300405	NOEC: 1000 mg CGA300405/kg soil	0.022	45 454	
	CGA275537[b]	2.52 mg a.s./kg dw soil	0.016	140	

[a,b] It is asummed that metabolites are up to 10 times more toxic than parental compound trinexapac-ethyl.

These long-term TER values exceed the Commission Regulation (EU) No. 546/2011 long-term trigger value of 5, indicating that the long-term risk to these soil fauna is acceptable following use of A8587F according to the proposed use pattern.

Conclusion: the acute and long-term TER values for the non-target soil meso-and macrofauna for the parent trinexapax-ethyl and its soil metabolite, and for the formulation are higher than the respective trigger values of 5 indicating an acceptable risk for the intended use of the formulation trinexapac-ethyl 250 g/L ME in cereals.

Soil Nitrogen Transformation

The risk assessment for effects on soil nitrogen transformation has been conducted according to the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10239/2002)
The risk to soil micro-organisms was evaluated by comparison of <25% effect levels with PEC_S values, as presented in the table below.

Table 2.9.9-27: Risk assessment for effects on soil micro-organisms

Test item	NOEC (mg a.s./kg)	PEC_S (mg/kg)
A8587F[a]	No unacceptable effect >25% on nitrification and respiration by day 28 at 10.7 mg A8587B/kg dry soil	0.807
Trinexapac-ethyl	No unacceptable effect >25% on nitrification and respiration by day 28 at 8.6 mg a.s./kg dry soil	0.213
Trinexapac (CGA179500) [b]	No data available, not considered necessary or alternatively NOEC: 0.86 mg/kg soi l	0.179
CGA300405	No effect >25% on nitrification by day 28 at 200 mg CGA300405/kg dry soil	0.022
CGA275537[c]	No unacceptable effect >25% on nitrification and respiration by day 28 at 0.86 mg a.s./kg dry soil	0.016

[ba] Tested as A8587B.
[b, c] It is asummed that metasbolites are up to 10 times more toxic than parental compound trinexapac-ethyl.

A8587F had no unacceptable effects on soil micro-organisms at 10.7 mg A8587F/kg. This is approximately 13 times higher than the maximum PEC_S of 0.807 mg A8587F/kg following the worst-case application to cereals. This indicates that the risk to non-target soil micro-organisms following use of A8587F according to the proposed use pattern is acceptable.

Furthermore, trinexapac-ethyl had no unacceptable effects on soil microorganisms at 2.6 mg a.s./kg. This is more than 12 times higher than the maximum PECs of 0.213 mg a.s./kg, indicating an acceptable risk to soil microorganisms.

For CGA179500 the estimated NOEL for soil microorganisms is 0.86 mg/kg. This is 4.8 times higher than the maximum PECs of 0.179 mg/kg, indicating an acceptable risk to soil microorganisms. CGA300405 had no unacceptable effects on soil micro-organisms at 200 mg CGA300405/kg. This is approximately 9000 times higher than the maximum PEC_S of 0.022 mg CGA300405/kg indicating an acceptable risk to soil microorganisms.

For CGA275537 the estimated NOEL for soil microorganisms is 0.86 mg/kg. This is 54 times higher than the maximum PECs of 0.016 mg/kg, indicating an acceptable risk to soil microorganisms.

Conclusion: When applied in accordance with the uses supported in this submission, A8587F poses an acceptable risk to soil microorganisms.

Terrestrial Non-Target Higher Plants

The risk assessment for effects on non-target higher plants has been conducted according to the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10239/2002)
Taking the lowest EC_{50} of >0.38 kg a.s./ha for pre- and post-emergence effects due to A8587F, and comparing to the PER of 0.00554 kg a.s./ha results in a TER of 69. This is well above the trigger value of 5 and indicates no unacceptable effects to off-field non-target plants following proposed uses of A8587F.

Conclusion: A8587F poses negligible risk to terrestrial non-target plants in off-crop areas.

Other terrestrial organisms (flora and fauna)

No data available

Biological methods for sewage treatment

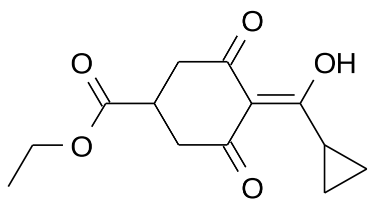
The risk to biological methods for sewage treatment is considered acceptable. The EC_{50} produced in the activated sewage sludge test (Grade, 2001) was greater than 100 mg a.s./L. The EC_{50} is > 1500 times greater than the FOCUS step 1 initial PECSW (63.6 µg/L). This suggests low risk to sewage treatment facilities.

2.10 Proposed harmonised classification and labelling according to the CLP criteria

2.10.1 Identity of the substance

2.10.1.1 Name and other identifiers of the substance

Table 76: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*IUPAC name:* 4-(cyclopropyl-hydroxymethylene)-3,5-dioxo-cyclohexanecarboxylic acid ethylester or ethyl (RS)-4-cyclopropyl(hydroxyl)methylene-3,5-dioxo-cyclohexanecarboxylate *CA name:* 4-(cyclopropyl-hydroxymethylene)-3,5-dioxo-cyclohexanecarboxylic acid ethylester or ethyl 4-(cyclopropylhydroxymethylene)-3,5-dioxocyclohexanecarboxylate
Other names (usual name, trade name, abbreviation)	trinexapac
ISO common name (if available and appropriate)	trinexapac-ethyl
EC number (if available and appropriate)	none allocated
EC name (if available and appropriate)	trinexapac-ethyl
CAS number (if available)	95266-40-3
Other identity code (if available)	manufacturer's development code number: CGA 163935 This code is given by the notifier Syngenta.
Molecular formula	$C_{13}H_{16}O_5$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	252.3 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	not applicable (not UVCB substance)
Degree of purity (%) (if relevant for the entry in Annex VI)	min. 950 g/kg

2.10.1.2 Composition of the substance

Table 77: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
trinexapac-ethyl	950 g/kg minimum purity of the technical grade active substance	-	Aquatic chronic 1; H410; M=1. GHS09; H410

Table 78: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
toluene CAS No. 108-88-3	max. 3 g/kg	Flam. Liq. 2, H225 Skin Irrit. 2, H315 Asp. Tox. 1, H304 STOT SE 3, H336 STOT RE 2, H373 Repr.2, H361d		No
ethyl (1*RS*)-ethyl 3-hydroxy-5-oxocyclohex-3-ene-1-carboxylate (CGA158377) CAS No. 88805-65-6	max 6 g/kg	Skin Irrit.2, H315 Eye Dam. 1, H318 Skin Sens. 1, H317		Possible contribution, however it is present at less than generic concentration limit 1.0% that trigger classification of a mixture

The impurities toluene and ethyl (1*RS*)-ethyl 3-hydroxy-5-oxocyclohex-3-ene-1-carboxylate (CGA158377) are considered relevant based on their hazard (reproductive toxicity and skin sensitisation, respectively).

Toluene was not detected in the technical material used in the skin sensitisation, three genotoxicity (old gene mutation *in vitro* and micronucleus *in vivo*), one critical (13-week rat), developmental, in part of chronic/carcinogenicity toxicity studies. The proposed specification for this impurity was above the values found in the technical material used in reproduction, three genotoxicity (old Ames and chromosome aberration *in vitro*), in part of one critical (1-year dog), in part of long-term toxicity/carcinogenicity, in the immunotoxicity, phototoxicity and neurotoxicity studies.

The applicant has submitted the Local lymph node assay (Anonymous, *2017* (B.6.2.6. Study 3)) with spiked batch material (Batch No SMO5D180_Fortified) to address skin sensitisation potential. The positive response was observed in the Local lymph node assay and it was concluded that trinexapac-ethyl (fortified) fulfilled the criteria for classification Skin Sens. 1B, H317 under the conditions of this study.

Table 79: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
not relevant	-	-	-	-	-

Table 80: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
trinexapac-ethyl technical grade active substance	950 g/kg			

2.10.2 Proposed harmonized classification and labelling

2.10.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 81: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	607-RST-VW-Y	trinexapac-ethyl (ISO) ethyl 4-[cyclopropyl(hydroxy)methylene]-3,5-dioxocyclohexanecarboxylate	-	95266-40-3	Skin Sens. 1B Aquatic chronic 1	H317 H410	GHS07 GHS09 Wng	H317 H410		M=1	
Resulting Annex VI entry if agreed by RAC and COM	607-RST-VW-Y	trinexapac-ethyl (ISO) ethyl 4-[cyclopropyl(hydroxy)methylene]-3,5-dioxocyclohexanecarboxylate	-	95266-40-3	Skin Sens. 1B Aquatic chronic 1	H317 H410	GHS07 GHS09 Wng	H317 H410		M=1	

2.10.2.2 Additional hazard statements / labelling

Table 82: Reason for not proposing harmonised classification and status under CLH public consultation

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Explosives	Hazard class not applicable	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	Yes
Oxidising gases	Hazard class not applicable	Yes
Gases under pressure	Hazard class not applicable	Yes
Flammable liquids	Hazard class not applicable	Yes
Flammable solids	Hazard class not applicable	Yes
Self-reactive substances	Conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Hazard class not applicable	Yes
Pyrophoric solids	Hazard class not applicable	Yes
Self-heating substances	Hazard class not applicable	Yes
Substances which in contact with water emit flammable gases	Hazard class not applicable	Yes
Oxidising liquids	Hazard class not applicable	Yes
Oxidising solids	Conclusive but not sufficient for classification	Yes
Organic peroxides	Hazard class not applicable	Yes
Corrosive to metals	Hazard class not applicable	Yes
Acute toxicity via oral route	Conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Conclusive but not sufficient for classification	Yes
Respiratory sensitisation	No data	No
Skin sensitisation	Skin Sens. 1B	Yes
Germ cell mutagenicity	Conclusive but not sufficient for classification	Yes
Carcinogenicity	Conclusive but not sufficient for classification	Yes
Reproductive toxicity	Conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Conclusive but not sufficient for classification	Yes

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Aspiration hazard	No data	No
Hazardous to the aquatic environment	H410, Chronic M-factor = 1	Yes
Hazardous to the ozone layer	Hazard class not applicable	No

2.10.3 History of the previous classification and labelling

No previous classification and labelling agreed.

2.10.4 Identified uses

Trinexapac-ethyl is proposed for use as plant growth regulator in the EU.

2.10.5 Data sources

Trinexapac-ethyl RAR prepared by Lithuania, Volumes 2 and 3, 2016

2.11 Relevance of metabolites in groundwater

2.11.1 STEP 1: Exclusion of degradation products of no concern

No metabolites excluded for this reason.

2.11.2 STEP 2: Quantification of potential groundwater contamination

PECgw values for trinexapac acid, CGA300405 and CGA275537 were below the trigger value of 0.1 µg/l in all FOCUS scenarios. Consequently, further assessment of the potential relevance of these compounds is not required.

2.11.3 STEP 3: Hazard assessment – identification of relevant metabolites

2.11.3.1 STEP 3, Stage 1: screening for biological activity

2.11.3.2 STEP 3, Stage 2: screening for genotoxicity

2.11.3.3 STEP 3, Stage 3: screening for toxicity

2.11.4 STEP 4: Exposure assessment – threshold of concern approach

2.11.5 STEP 5: Refined risk assessment

2.11.6 Overall conclusion

2.12 Consideration of isomeric composition in the risk assessment

Considered in the risk assessment.

2.12.1 Identity and physical chemical properties

2.12.2 Methods of analysis

2.12.3 Mammalian toxicity

2.12.4 Operator, Worker, Bystander and Resident exposure

2.12.5 Residues and Consumer risk assessment

2.12.6 Environmental fate

2.12.7 Ecotoxicology

2.13 Residue definitions

2.13.1 Definition of residues for exposure/risk assessment

Food of plant origin*:*

- *trinexapac, free and conjugated (cereal grain)(provisional)*
- *trinexapac, free and conjugated plus CGA300405 (cereal fodder items/grass) (expressed as trinexapac or separate, pending its toxicological relevance) (provisional)*

Food of animal origin*:*

- *• Poultry : trinexapac*
- *• Ruminant: trinexapac plus metabolite CGA 113745, expressed as trinexapac (Provisional), pending the outcome of the cyclopropyl label metabolism study*

Soil: *trinexapac ethyl, trinexapac acid, CGA300405, CGA275537*

Groundwater*: trinexapac ethyl, trinexapac acid, CGA300405, CGA275537*

Surface water*: trinexapac ethyl, trinexapac acid, CGA300405, CGA275537, M2 (3 carboxylic acid ethyl ester-7-hydroxypropyl-5-oxo,7-hydroxyheptanoic acid), WaterM3Photolysis*

Sediment*:-*

Air: *trinexapac ethyl*

2.13.2 Definition of residues for monitoring

Food of plant origin*: sum of trinexapac and its salts, expressed as trinexapac (cereal/grass)*

Food of animal origin*: sum of trinexapac and its salts, expressed as trinexapax*

Soil: *trinexapac ethyl*

Groundwater*: trinexapac ethyl*

Surface water*: trinexapac ethyl*

Sediment*: trinexapac ethyl*

Air*: trinexapac ethyl*

LEVEL 3

Summary and consideration with respect to the approval criteria of Regulation (EC) No 1107/2009
Identification of data gaps, proposed conditions, risk management measures, issues that could not be finalised and critical areas of concern
Proposed decision

3 Proposed decision with respect to the application of approval or renewal of the approval of an active substance

3.1 Background to the proposed decision

3.1.1 Proposal on acceptability against the approval criteria – Article 4 and Annex II of Regulation (EC) No 1107/2009

3.1.1.1 Article 4				
		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.			
3.1.1.2 Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted	X		A complete dossier has been submitted to allow the conduct of a comprehensive risk assessment but additional information and expert consultation is considered necessary by Regulation. Please refer to 3.1.4 “List of studies to be generated, still ongoing or available but not evaluated” and 3.1.8 “Area(s) where expert consultation is considered necessary”.

ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			
3.1.1.3 Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.			
3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		The data submitted are sufficient to establish an Acceptable Daily Intake (ADI) and Acceptable Operator Exposure Level (AOEL). Results from the toxicological studies do not raise the need for setting an Acute Reference Dose (ARfD).
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level			

	reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.			
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.			
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.			
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.		X	Data gap identified for the repeated exposure toxicity (available 90-day rat study to JMPR) and updated literature search regarding metabolite CGA224439.
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern		X	Insufficient information has been available to support the proposed technical specification of trinexapac-ethyl with respect to the identity and content of impurities in the specification.

	within acceptable limits.			
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.	-	-	Not applicable. FAO specification has not been allocated for trinexapac-ethyl
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted	-	-	Not applicable. FAO specification has not been allocated for trinexapac-ethyl
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.	X		The Regulation (EU) 283/2013 on data requirements for active substances, Part A Section 4, 4.2 (d) *Methods for post-approval control and monitoring purposes* stipulate that *Methods, with a full description , have been submitted for:* *(d) the analysis in body fluids and tissues for active substances and relevant metabolites.*
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	

	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		See Vol 1, Level 2, sections 2.6.10.1 (ADI), 2.6.10.2 (ARfD) and 2.6.10.3 (AOEL). The ADI of 0.32 mg/kg bw/day determined from the one-year oral toxicity study in dogs. Results from the toxicological studies do not raise the need for setting an Acute Reference Dose (ARfD). The AOEL of 0.34 mg/kg bw/day determined from the 90-day study in rat. No correction factor for oral absorption is considered necessary.
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	Overall, the results do not indicate that trinexapac-ethyl possesses a genotoxic potential in vivo.
Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**		X	Overall, the results do not indicate sufficient evidence that trinexapac-ethyl possesses a carcinogenic potential.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic	-	-	Not applicable

	proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**		X	There was no sufficient evidence of reproductive and developmental toxicity of trinexapac-ethyl investigated in a two-generation reproduction study in rats and developmental studies in rats and rabbits.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.	-	-	Not applicable
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties**		X	No sufficient evidence of carcinogenicity or reproductive toxicity was seen in the standard carcinogenicity and reproductive toxicity studies.

ii)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **toxic for reproduction category 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**		X	No sufficient evidence of reproductive toxicity was seen in the standard reproductive toxicity studies.
iii)	Linked to either i) or ii) immediately above. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.	-	-	Not applicable
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	All results for trinexapac-ethyl are below these criteria.
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	All results for trinexapac-ethyl are below these criteria.
Very persistent and very bioaccumulative substance (vPvB).				

		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	All results for trinexapac-ethyl are below these criteria.
Ecotoxicology				
		Yes	No	
i)	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.	X		The acute and reproductive risks to birds and mammals are shown to be acceptable at the screening step and Tier 1 for all proposed uses. The risk to aquatic organisms for all groups of organisms is resolved on Step 1 and 2. Acceptable risks to bees and other non-target arthropods are demonstrated at first tier. Low risks to soil organisms are also demonstrated. The risk to terrestrial non-target plants is resolved. A low risk to microorganisms in sewage is concluded. The above applies to all representative uses (see sections B.9.1 to B.9.14 of Volume 3 (PPP) for further details).
ii)	It is considered that, on the basis of the assessment of Community or internationally agreed test guidelines, the substance **HAS** endocrine disrupting properties that may cause adverse effects on non-target organisms.		X	Based on the mammalian toxicology assessment, trinexapac-ethyl is not considered an endocrine disrupter and does not meet the interim criteria for this currently established in Regulation 1107/2009. The applicant has proposed that the active substance does not have endocrine disrupting properties.
iii)	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.	X		Trinexapac-ethyl is not considered an endocrine disrupter.
iv)	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or	X		The risk assessment for honey bees (*Apis melifera*) indicated an acceptable risk based on first tier assessment (see Volume 1, Level 2, section 2.9.9.3.1 for the risk assessment summary). The risk was acceptable for all the representative uses and products considered by the assessment. The risk assessment was conducted according to SANCO/10329/2002, the

	synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.			guidance available at the time of the assessment. Therefore, no formal consideration of effects on colony survival and development has been conducted, as this is not part of the SANCO/10329/2002 risk assessment procedure. Studies have been submitted and evaluated, investigating semi-chronic larval and chronic-adult toxicity, in line with the data requirements. In order to take all available data into account, an assessment following the revised EPPO guideline for bees (2010) was performed for the chronic risk to adult honeybees and honeybee larvae. This assessment did not indicate an unacceptable chronic risk to honeybees. Further, an assessment for the chronic risk to adult honeybees and honeybee larvae was performed according to the EFSA guidance document for bees (2013). On this basis there is no evidence to suggest an unacceptable risk to colony survival and development due to chronic or acute effects of the product/active substance.
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.		X	At the expert meeting (PPR 171, 13 – 15 December 2017) it was agreed that in view of the pending data request for a new metabolism study in cereals with the cyclopropyl label and further clarification on metabolites, the RD for RA for primary crops - the cereal/grass crop category, rotational crops and animal commodities could be proposed as provisional only. To address the effect of food processing conditions on residues, four standard hydrolysis studies were submitted showing partially contradictory outcomes. Two studies were suggesting the stability of trinexapac-ethyl and trinexapac, respectively under hydrolysis conditions while the other two studies showed significant degradation under baking and sterilisation conditions. The experts were unable to conclude on the relevant residues in processed commodities. Further clarification by the applicant to explain the ambiguous findings in this standardised experiment is necessary. Also a data gap was set - further clarification should be submitted by the applicant to explain the contradictory findings (stability vs. instability) in the

				standardised hydrolysis experiments. Residue definition for processed commodities could not be proposed. In addition, the lack of information on metabolites CGA313458, CGA 113745 and CGA224439 was identified. CGA313458 – unclear amounts in bran, flour and bread due to storage stability. CGA113745 – unclear amounts in all processed commodities due to storage stability. CGA224439 – risk assessment could not be finalised due to lack of reliable reference values.
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.			

3.1.2 Proposal - Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	Trinexapac-ethyl does not fulfil any of the criteria for identification of candidates for substitution

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** In particular it is considered that the substance **should NOT be classified or proposed for classification** in accordance with Regulation (EC) No 1272/2008 as at least one of the following: — carcinogenic, — mutagenic, — toxic to reproduction, — sensitising chemicals, — very toxic or toxic, — explosive, — corrosive. In addition it is considered that **the substance is NOT**: — persistent (half-life in soil more than 60 days), — has a bioconcentration factor higher than 100, — is deemed to be an endocrine disrupter, or — has neurotoxic or immunotoxic effects.		X	May not be regarded as low risk because of proposal for harmonised classification according to Regulation (EC) No 1272/2008: Skin Sens. 1B, H317 May not be regarded as low risk because of proposal for harmonised clasification according to Regulation (EC) No 1272/2008: H410; Very toxic to aquatic life with long lasting effects.

3.1.4 List of studies to be generated, still ongoing or available but not evaluated

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	**Study on-going and anticipated date of completion**	**Study available but not peer-reviewed**
3.1.4.1 Identity of the active substance or formulation				
Syngenta: Report of the analysis of the impurity profile of the ecotoxicological batch: SMO4D0962	relevant for all uses	X		
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
3.1.4.3 Data on uses and efficacy				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
3.1.4.5 Methods of analysis				

Considering the relevance of impurities confirmed the method for formulation analysis for their determination is required	relevant for all uses	X		
3.1.4.6 Toxicology and metabolism				
No comparative *in vitro* dog and human metabolism study is available.	relevant for all uses	X		
Syngenta: Further data are needed to exclude the relevance of some impurities. Data required are listed in the confidential *Vol 4 Syngenta C.1.4.2*.	relevant for all uses	X		
Adverse effect of trinexapac-ethyl on the oestrus cycle in 1-year dog study needs to be further addressed and further clarification of the ED potential using additional mechanistic data is requested. At the expert meeting (PPR 170, 11 – 14 December 2017), the majority of experts suggested to provide *in vitro* assays (e.g. Steroidogenesis assay, OECD TG 456)	relevant for all uses	X		
At the expert meeting (PPR 170, 11 – 14 December 2017), the data gap was proposed to address the repeated exposure toxicity (available 90-day rat study to JMPR) and updated literature search of the metabolite CGA224439.	relevant for all uses	X		
3.1.4.7 Residue data				
Study on magnitude of residues in honey.	Relevant for all uses		First quarter 2018	
Study on magnitude of metabolites CGA313458 and CGA113745 in processed commodities	Relevant for all uses	X		

(covered by storage stability).				
Storage stability study for trinexapac acid in cereal grains and straw covering the maximum length of storage of the samples from the residue trials.	Relevant for all uses		First quarter 2019	
Storage stability study for metabolite CGA113745 in processed commodities, analysing samples in smaller steps during the first month of storage.	Relevant for all uses	X		
3.1.4.8 Environmental fate and behaviour				
3.1.4.9 Ecotoxicology				

3.1.5 Issues that could not be finalized

Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**
The suitability of the mammalian toxicology tested batches of the technical a.s. to support the proposed specification of trinexapac-ethyl.	Relevant for all representative uses
Proposed residue definition for plants (primary and rotational crops, animal and processed commodities for monitoring and risk assessment could not be finalised pending data request for a new metabolism study in cereals with the cyclopropyl label and further clarification on metabolites.	Relevant for all representative uses.
Risk assessment for consumers could not be finalised due to provisional residue definitions.	Relevant for all representative uses.

3.1.6 Critical areas of concern

Critical area of concern identified	**Relevance in relation to representative use(s)**
The technical specification is not covered by the (eco)toxicological assessment	Relevant for all representative uses

3.1.7 Overview table of the concerns identified for each representative use considered

Representative use		Use "A" (X[1])	Use "B" (X[1])
Operator risk	Risk identified		
	Assessment not finalised		
Worker risk	Risk identified		
	Assessment not finalised		
Bystander risk	Risk identified		
	Assessment not finalised		
Consumer risk	Risk identified		
	Assessment not finalised	X	X
Risk to wild non target terrestrial vertebrates	Risk identified		
	Assessment not finalised		
Risk to wild non	Risk identified		

target terrestrial organisms other than vertebrates	Assessment not finalised		
Risk to aquatic organisms	Risk identified		
	Assessment not finalised		
Groundwater exposure active substance	Legal parametric value breached		
	Assessment not finalised		
Groundwater exposure metabolites	Legal parametric value breached		
	Parametric value of 10µg/L[(a)] breached		
	Assessment not finalised		
Comments/Remarks			

1) The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

Area(s) where expert consultation is considered necessary	Justification

3.1.9 Critical issues on which the Co-RMS did not agree with the assessment by the RMS

Issue on which Co-RMS disagrees with RMS	Opinion of Co-RMS	Opinion of RMS
None		

3.2 Proposed decision

3.3 Rational for the conditions and restrictions to be associated with any approval or authorisation(s), as appropriate

3.3.1 Particular conditions proposed to be take into account to manage the risks identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
None	

APPENDICES

Appendix 1 Guidance documents used in this assessment

European Commission, 2000a. Residues: guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414. SANCO/3029/99-rev. 4, 11 July 2000

European Commission, 2000b. Technical material and preparations: guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414. SANCO/3030/99-rev. 4, 11 July 2000

European Commission, 2010. Guidance Document on residue analytical methods. SANCO/825/00-rev. 8.1, 16 November 2010

European Commission, 2012. Guidance document on the assessment of the equivalence of technical materials of substances regulated under Regulation (EC) No 1107/2009. SANCO/10597/2003-rev. 10.1, 13 July 2012.

European Commission, 2014. Guidance document on the renewal of approval of active substances to be assessed in compliance with Regulation (EU) No 844/2012. SANCO/2012/11251-rev. 4, 12 December 2014.

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EFSA, 2009; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA, EFSA Journal 2009; 7(12):1438.

EFSA, 2012. Guidance on Dermal Absorption, EFSA Journal 2012; 10(4):2665

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Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329/2002, rev 2 (final) 17 October 2002.

OECD, 2007. Guideline for the testing of chemicals. 501 Metabolism in crops. 20 pp.

OECD, 2007. Guideline for the testing of chemicals. 502 Metabolism in rotational crops. 18 pp.

OECD, 2007. Guideline for the testing of chemicals. 503 Metabolism in livestock. 21 pp.

OECD, 2007. Guideline for the testing of chemicals. 504 Residues in rotational crops (Limited field studies). 9 pp.

OECD, 2007. Guideline for the testing of chemicals. 505 Residues in livestock. 21 pp.

OECD, 2007. Guideline for the testing of chemicals. 506 Stability of pesticide residues in stored commodities. 12 pp.

OECD, 2007. Guideline for the testing of chemicals. 507 Nature of the pesticide residues in processed commodities – high temperature hydrolysis. 15 pp.

OECD, 2008. Guideline for the testing of chemicals. 508 Magnitude of the pesticide residues in processed commodities. 15 pp.

OECD, 2008. Guidance document on magnitude of pesticide residues in processed commodities, Series on Testing and Assessment No. 96. ENV/JM/MONO(2008)23, 44 pp.

OECD, 2009. Guidance document on the definition of residue, Series on Pesticides No. 31, Series on Testing and Assessment No. 63. ENV/JM/MONO(2009)30, 38 pp.

OECD, 2009. Guideline for the testing of chemicals. 509 Crop field trial. 44 pp.

OECD, 2013. Guidance document on residues in livestock, Series on Pesticides No.73. ENV/JM/MONO(2013)8, 77pp.

OECD, 2016. Guidance document on crop field trials, Series on Pesticides No. 66. Series on Testing and Assessment No. 164. ENV/JM/MONO(2011)50/REV1, 43 pp.

FOCUS, 2000. "FOCUS groundwater scenarios in the EU review of active substances" Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference SANCO/321/2000 rev.2, 202pp

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Appendix 2 Reference list

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WHO/FAO, 2013. Pesticide residues in food - 2013: evaluations / Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Geneva, Switzerland, 17–26 September 2013. Part 1 - Residues, pp 1595 – 1716.

WHO/FAO, 2013. Pesticide residues in food - 2013: toxicological evaluations / Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Geneva, Switzerland, 17–26 September 2013. Part 2, Toxicological, pp 553 – 580.